OPERATOR'S MANUAL

BE2x00

Noninvasive Biomass Monitor

User manual for the following BugLab products:
BE2100 sensor
BE2100 base unit (single sensor)
BE2400 base unit (4-sensor multiplexing)
BE|USB adapter

Includes Instructions for BE2x00 Virtual Instrument and Data Viewing Software

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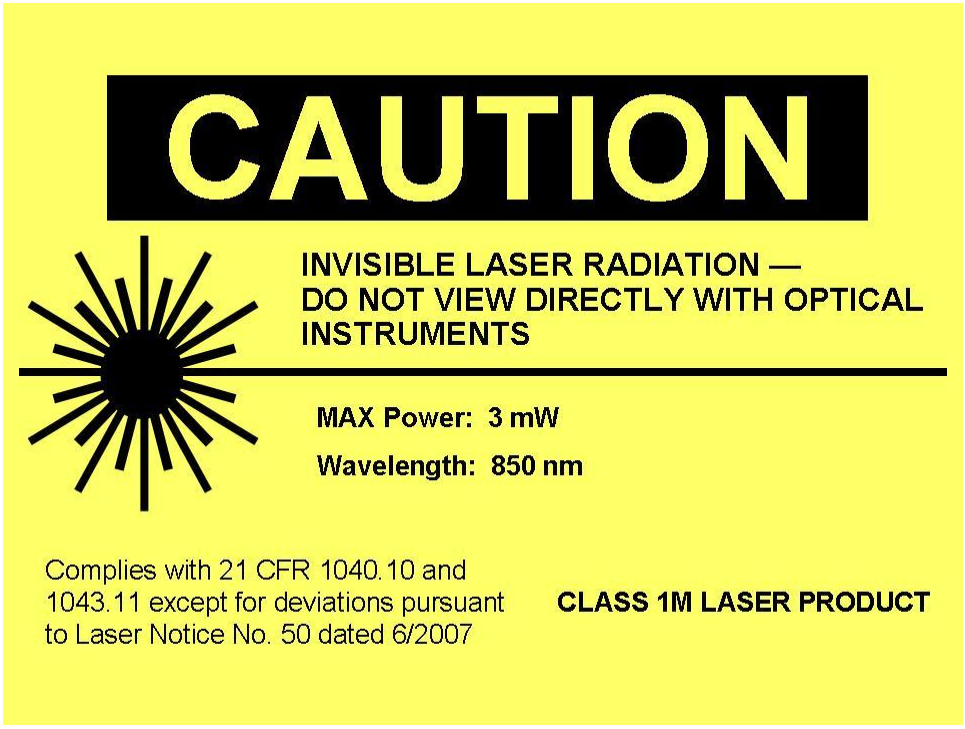
Cautions


CAUTION
INVISIBLE LASER RADIATION —
DO NOT VIEW DIRECTLY WITH OPTICAL
INSTRUMENTS
MAX Power: 3 mW
Wavelength: 850 nm
Complies with 21 CFR 1040.10 and
1043.11 except for deviations pursuant
to Laser Notice No. 50 dated 6/2007
CLASS 1M LASER PRODUCT

Viewing the laser output with certain optical instruments (for example, eye loupes, magnifiers and microscopes) may pose an eye hazard.

The low-voltage BE2100 sensor is water-resistant, but it is not water-proof. Keep the sensor, the Base Unit, the cables and power supply clean and dry.

Do not immerse the optical sensor.

Do not drop or shake the sensor.

Do not expose the sensor to direct sunlight or high temperatures.

Remove the sensor during any type of bioreactor autoclave cycle.

There are no user-serviceable parts inside the sensor or the main unit.

<u>Do not leave fingerprints or dirt on the optical sensor surface</u>. The sensor is a precision optical device. It may be cleaned with a light application of commercial window cleaner, ethanol, or isopropanol and gentle wiping with paper or cloth towels. Optical grade lens cleaning tissue is preferred. (Do not use acetone to clean the sensor. Some of the materials used in the sensor will rapidly degrade if exposed to acetone.)

<u>Important note to users with more than one BE2100 sensor</u>: The calibration cups provided for testing sensor performance are not identical and should be matched with the corresponding sensor. The last 2 digits of the serial numbers printed on the sensor and low and high calibration cups should all match each other.

<u>Important note to BE2x00 software users</u>: Before plugging the BE2100 into your computer, make sure you have installed the software first. Configure the power settings on your computer to never go into sleep mode.

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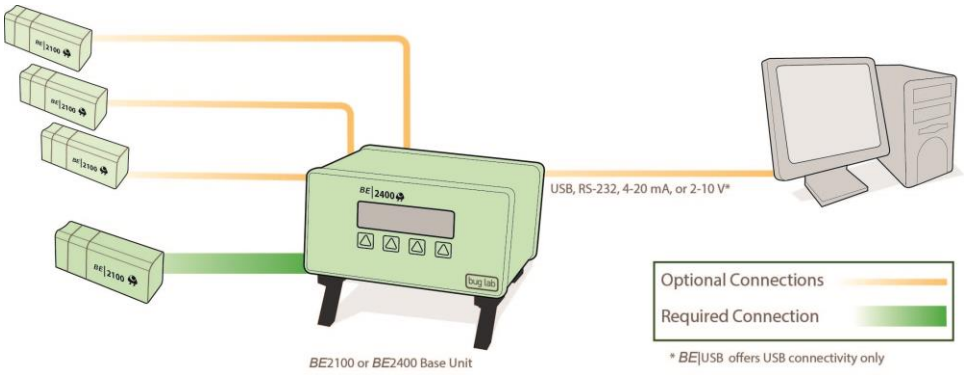
INTRODUCTION

This User Manual describes the operation of the BE2100 non-invasive biomass sensor. The sensor measures biomass in liquid cultures using laser optical reflectance at 850 nm. The optical reflectance is measured through the vessel wall of glass fermenters or through transparent port windows provided in stainless steel bioreactors. The sensor must be placed below the liquid-air interface in order to provide accurate measurements. Calibration tools are provided so that results can be reported in whatever biomass units are desired, such as dry cell weight (g/L), optical density (OD), or cell density (cells/mL). Specific calibration to the particular type of organism being measured is recommended. Tools are also provided for baseline correction of media reflectance, as measured in the absence of biomass.

There are currently three options for connecting to the BE2100 sensor, as depicted in the Figure on the next page (these different options are collectively referred to in this manual as the "BE2x00" instrument). In the standard configuration, the BE2100 sensor is connected to a BE2100 base unit and operated either in conjunction with a personal computer (PC) or in stand-alone mode. The B2100 base unit provides a connection for one BE2100 sensor. The sensor configuration may be set up either through a keypad interface or via the provided Virtual Instrument software. Biomass results are visually displayed on the BE2100 base unit screen, and are also available in digital (RS-232 or USB) and analog (4-20 mA, or 2-10 V) format. The BE2400 base unit offers the same functionality as a BE2100 base unit, but with the ability to connect to up to 4 BE2100 sensors. A third sensor connectivity option is the BE|USB adapter. This small adapter allows the BE2100 sensor to be plugged directly into a PC. With this option all configuration setting and data communications are performed via the PC.

The BE2x00 Virtual Instrument software provided with the instrument works with all of the above-described hardware configurations. Configuration of, calibration of, and data collection from up to six sensors can be simultaneously performed from within the same program window. At program start-up, a search is automatically performed for all connected BE2100 sensors. All configuration settings are displayed in tables, and the settings can be modified with a simple mouse click. Data acquisition is graphically displayed in separate tabbed windows for each sensor. Important events that occur during the bioreactor run can be marked directly on the graph, and are displayed in a summary table for each sensor. A combined graph window also allows the results for all sensors to be overlaid. A separate BE2x00 Data Viewing program allows previously acquired data to be viewed, manipulated, and resaved. Detailed instructions for operating both the BE2x00 Virtual Instrument and Data Viewing software are provided in Section 2 of this manual.


USB, RS-232, 4-20 mA, or 2-10 V*
Optional Connections
Required Connection
* BE|USB offers USB connectivity only
BE2100 or BE2400 Base Unit

GETTING STARTED

Unpacking the Instrument

The BE2100 sensor consists of:

Optical Sensor Head with cable (2 m)
Sensor Adapter Sleeves (10, 15, and 20mm)
Calibration Cups ("Low" and "High")

Additional components provided with a BE2100 base unit:

BE2100 Base Unit
Power Adapter
RS-232 and USB cables (2 m)
Analog output terminal block (4-position)
Resistors (500 Ω) for optionally converting 4-20 mA analog outputs into 2-10 V outputs.
BE2x00 Virtual Instrument and Data Viewing Software

Additional components provided with a BE2400 base unit:

BE2400 Base Unit
Power Adapter
RS-232 and USB cables (2 m)
Analog output terminal block (8-position)
Resistors (500 Ω) for optionally converting 4-20 mA analog outputs into 2-10 V outputs.
BE2x00 Virtual Instrument and Data Viewing Software

Additional components provided with a BE|USB adapter:

BE|USB adapter
USB cable (2 m)
BE2x00 Virtual Instrument and Data Viewing Software

Optional accessories:

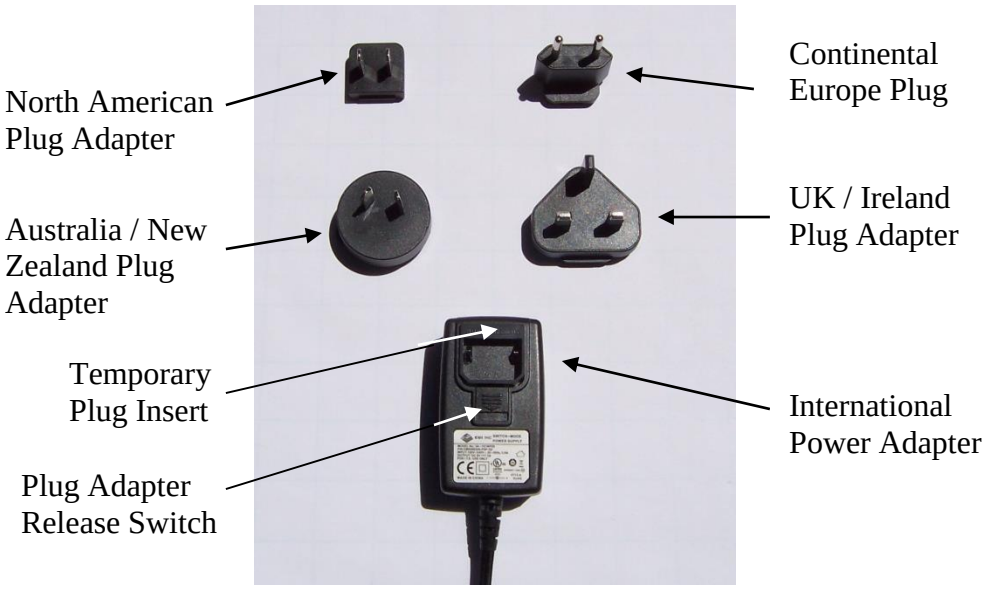
Extended (7.5 m) RS-232 cable
Extended (5 m) USB cable
Screw-mounting Sensor Attachment Kit
Sensor Adapter Sleeve (5 mm)
Sensor cable extender (4 m)
Sensor strap extender (2 m)

Note: Unpack and inspect all of the components to assure that they have not been damaged in shipping.

Note: The BE2100 is an optical instrument and will only operate on transparent cylindrical glass bioreactors or bioreactors with a glass viewing port that makes contact with the fermentation solution.

Configuring the Power Adapter for BE2100 and BE2400 Base Units (International Version only)

Note: When sensor connectivity is provided through a BE|USB adapter, power is provided to the sensor via the USB connection (ie. no separate power adapter is required).


North American Plug Adapter
Continental Europe Plug
Australia / New Zealand Plug Adapter
UK / Ireland Plug Adapter
Temporary Plug Insert
International Power Adapter
Plug Adapter Release Switch

STEP 1: Slide the Plug Adapter Release Switch into the release position (towards the power cord), and remove the temporary plug insert.

STEP 2: Select the Plug Adapter appropriate for the country in which the instrument is to be used and insert it while holding the Plug Adapter Release Switch in the release position.

STEP 3: Release the Plug Adapter Release Switch and check that the Plug Adapter is held securely in the power adapter.

Connecting the Sensor and Base Unit (or BE|USB Adapter)

STEP 1: Connect the sensor cable(s) to the rear of the Base Unit (or to the sensor connector on the BE|USB adapter). Make sure the 4 pins on the sensor connector match up with the 4 holes on the Base Unit (or BE|USB connector). Screw the connector in by hand until it reaches a stop. Do not use tools to further tighten the connector.

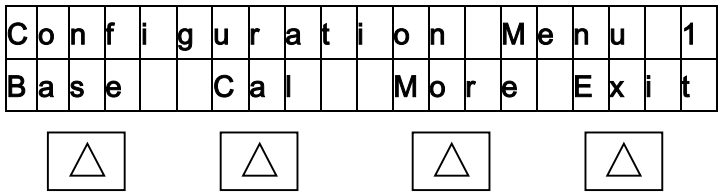
STEP 2: Connect the power adapter cable to the Base Unit (or the USB cable to the BE|USB adapter).

STEP 3: Plug the power adapter into a power source and switch the unit on (or plug the USB cable coming from the BE|USB adapter into your computer and the sensor will automatically be powered on).

Introduction to the BE2100 Base Unit Functions

The keypad interface on BE2100 and BE2400 base units provides access to many functions, a full description of which can be found in Appendix II. Because the BE2400 base unit connects to up to 4 sensors, the keypad interface is a little different than that of the BE2100. Skip to the next section ("Introduction to the BE2400 Base Unit Functions") if you are using a BE2400 base unit. All of the functions available via the keypad interface are also available through the Virtual Instrument Software. If you are using a BE|USB adapter or plan to operate the instrument solely through the Virtual Instrument software, you can skip this section. However, if you wish to operate a BE2100 base unit in stand-alone mode (without a computer), the following tutorial may be helpful. This tutorial will give you familiarity with the basic functioning of the keypad interface by leading you through the steps required to modify one parameter, the sensor response time:

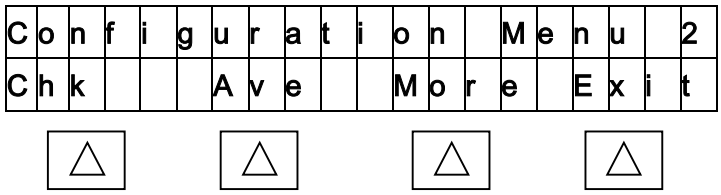
STEP 1: Press any of the 4 arrowhead- keys on the front of the Base Unit. The BE2100 base unit display should now look like:



Pressing the arrowhead below the words "Base" or "Cal" would lead you, respectively, into sub-menus controlling the Baseline and User Calibration features. Pressing the arrowhead beneath the word "Exit" would return you to the "normal" display screen.

Note: If you are not the 1st user of the instrument and a prior user has turned on password protection, before you can reach the above configuration menu you will be prompted to enter a password. You may wish to consult the prior user to obtain the password. Alternatively, if the password has been lost or forgotten, the password may be reset via the Virtual Instrument software. If this is the case, skip this section for now, and come back to it later after you have installed the user interface software and reset the keypad password.

STEP 2: Press the arrowhead beneath the word "More". The display should now look like:



Pressing the arrowhead below the word "Chk" would lead you into the "Sensor Check" function. This function allows you to verify sensor performance and if necessary update calibration coefficients using measurements made on the "Low" and "High" calibration cups.

STEP 3: Press the arrowhead beneath the word "Ave". The display should now look like:

T	i	m	e		C	o	n	s	t	a	n	t		S	e	l	e	c	t
	S	e	t		H	e	l	p		S	h	o	w			B	a	c	k

STEP 4: Press the arrowhead beneath the word "Help". A brief description of the function flashes onto the screen, after which the screen reverts to that shown above in Step 3.

STEP 5: Press the arrowhead beneath the word "Show". The currently selected time constant is shown, along with the default time constant.

STEP 6: Press the arrowhead beneath the word "Set". The display should now look like:

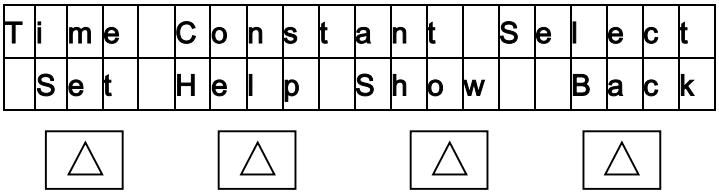
S	e	l	e	c	t		T	i	m	e		C	o	n	s	t	a	n	t
0	S	e	c		3	0	S	e	c		M	o	r	e		B	a	c	k

Pressing the arrowhead below the word "More" would lead you to menus with other choices for time constants. Time Constants between 0 seconds and 8 minutes can be selected.

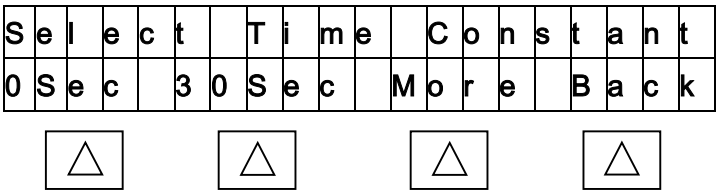
> **Note**: Use of the "0 sec" time constant is generally not advised, except when very fast sensor response is required, or as a temporary measure, such as when initially positioning the sensor on a crowded bioreactor vessel. When the time constant is set to 0 seconds, digital filtering is turned off, and only analog filtering (with a time constant of approximately 3 seconds) is applied to the reported sensor data.

STEP 7: Press the arrowhead beneath "30Sec". The screen will briefly show the new value that has been set. The new value is immediately saved into sensor memory, and will be recalled even if you switch the instrument off and on again. The screen then returns to the 2nd Configuration menu (as shown above in Step 2).

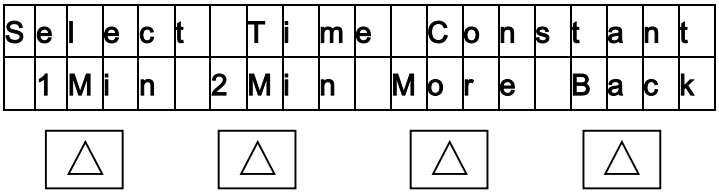
STEP 8: Press the arrowhead beneath the word "Ave" again. The display should now look like:



STEP 9: Press the arrowhead beneath the word "Set". The display should now look like:



STEP 10: Press the arrowhead beneath the word "More". The display should now look like:



STEP 11: Press the arrowhead beneath "2Min" (or select whatever time constant is most appropriate for your measurement conditions). The screen will briefly show the new value that has been set. The new value is immediately saved into sensor memory, and will be recalled even if you switch the instrument off and on again. The screen then returns to the 2nd Configuration menu (as shown above in Step 2).

STEP 12: Press the arrowhead beneath the word "More". Pressing the arrowheads beneath "AO1" and "AO2" would lead you into sub-menus that would allow you to set parameters associated with the 4-20 mA analog outputs available on the rear of the Base Unit. AO1 provides a signal that is proportional to the signal displayed on the front of the Base Unit (when not in the Configuration menus). AO2 provides a signal that is proportional growth rate (in 1/hours) of the AO1 signal. The correspondence

between the analog outputs and the signal values is determined by the range settings, available in these sub-menus. The time window over which AO2 is computed can also be selected within these sub-menus.

STEP 13: Press the arrowhead beneath the word "More". Pressing the arrowhead beneath "Comm" and "Disp" would lead you into sub-menus that would allow you to respectively change the communication settings and display brightness.

STEP 14: Press the arrowhead beneath the word "More". Pressing the arrowhead beneath "Pswd" would lead you into a sub-menu that allows you to password-restrict access to the keypad functions. Pressing the arrowhead beneath the word "Reset" would allow you to reset all parameters to their factory default values.

STEP 15: Press the arrowhead beneath the word "Exit". This will exit you out of the configuration menus and return you to the "normal" display window. Note that communication via the USB or RS-232 ports is disabled while the configuration menu is active, so make sure to exit the configuration windows before trying to run the BE2x00 Virtual Instrument software.

Now that you have a basic familiarity with the operation of the keypad interface, you may want to experiment with other features. Alternatively, a detailed step-by-step description of the keypad features is available in Appendix II. Once you have selected settings that are best for your application, you may wish to prevent accidental changes to these settings by turning on the password protection feature.

Introduction to the BE2400 Base Unit Functions

If you are using a BE2100 base unit, or a BE|USB adapter, or plan to operate the instrument solely through the Virtual Instrument software, you can skip this section. However, if you wish to operate a BE2400 base unit in stand-alone mode (without a computer), the following tutorial may be helpful. This tutorial will give you familiarity with the basic functioning of the keypad interface by leading you through the steps required to modify one parameter, the sensor response time:

STEP 1: Press any of the 4 arrowhead keys on the front of the Base Unit. The display should now look like:

C	o	n	f	i	g	u	r	a	t	i	o	n		M	e	n	u		1
S	c	r	o	l		L	o	c	k		M	o	r	e		E	x	i	t

The "Scrol(l)" and "Lock" options on the BE2400 base unit provide the ability to scroll the display through all available sensors (at 5 second intervals), or to lock the display to one particular sensor. The factory default setting is scrolling mode. Press the "More" button to access all other configuration options. The BE2400 base unit display should now look like:

S	e	l	e	c	t		s	e	n	s	o	r		n	u	m	b	e	r
		1					2					3					4		

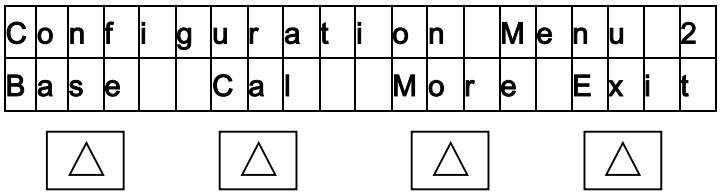
Note: if less than 4 sensors are plugged into the BE2400 base unit, only those sensor numbers that are plugged will be shown.

Note: If you are not the 1st user of the instrument and a prior user has turned on password protection, before you can reach the above configuration menu you will be prompted to enter a password. You may wish to consult the prior user to obtain the password. Alternatively, if the password has been lost or forgotten, the password may be reset via the Virtual Instrument software. If this is the case, skip this section for now, and come back to it later after you have installed the user interface software and reset the keypad password.

Select the arrowhead button below one of the sensor numbers.

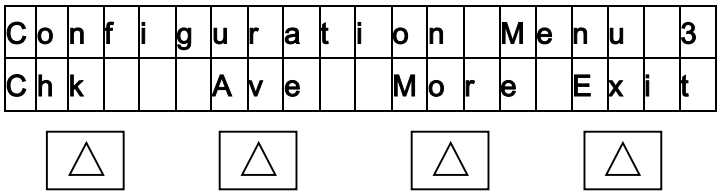
Note: In this exercise you will only be changing the time response for the sensor number that you select. If you wish to change settings for more than one sensor, each sensor attached to the BE2400 base unit must be individually configured.

The BE2400 base unit display should now look like:



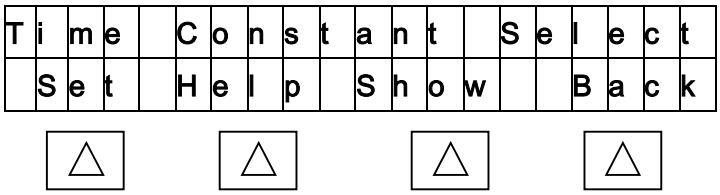
Pressing the arrows below the words “Base” or “Cal” would lead you, respectively, into sub-menus controlling the Baseline and User Calibration features. Pressing the arrowhead beneath the word “Exit” would return you to the “normal” display screen.

STEP 2: Press the arrowhead beneath the word “More”. The display should now look like:



Pressing the arrowhead below the word “Chk” would lead you into the “Sensor Check” function. This function allows you to verify sensor performance and if necessary update calibration coefficients using measurements made on the “Low” and “High” calibration cups.

STEP 3: Press the arrowhead beneath “Ave”. The display should now look like:



STEP 4: Press the arrowhead beneath the word “Help”. A brief description of the function flashes onto the screen, after which the screen reverts to that shown above in Step 3.

STEP 5: Press the arrowhead beneath the word “Show”. The currently selected time constant is shown, along with the default time constant.

STEP 6: Press the arrowhead beneath the word “Set”. The display should now look like:

S	e	l	e	c	t		T	i	m	e		C	o	n	s	t	a	n	t
0	S	e	c		3	0	S	e	c		M	o	r	e		B	a	c	k

△ △ △ △

Pressing the arrowhead below the word "More" would lead you to menus with other choices for time constants. Time Constants between 0 seconds and 8 minutes can be selected.

> **Note**: Use of the "0 sec" time constant is generally not advised, except when very fast sensor response is required, or as a temporary measure, such as when initially positioning the sensor on a crowded bioreactor vessel. When the time constant is set to 0 seconds digital filtering is turned off, and only analog filtering (with a time constant of approximately 3 seconds) is applied to the reported sensor data.

STEP 7: Press the arrowhead beneath the word "30Sec". The screen will briefly show the new value that has been set. The new value is immediately saved into sensor memory, and will be recalled even if you switch the instrument off and on again. The screen then returns to the 3rd Configuration menu (as shown above in Step 2).

STEP 8: Press the arrowhead beneath the word "Ave" again. The display should now look like:

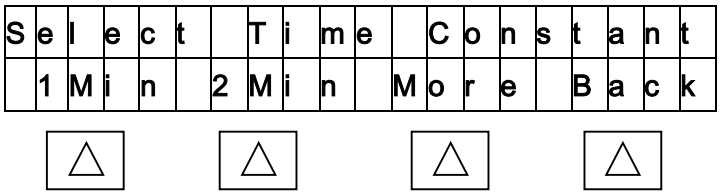
T	i	m	e		C	o	n	s	t	a	n	t		S	e	l	e	c	t
	S	e	t		H	e	l	p		S	h	o	w			B	a	c	k

△ △ △ △

STEP 9: Press the arrowhead beneath the word "Set". The display should now look like:

S	e	l	e	c	t		T	i	m	e		C	o	n	s	t	a	n	t
0	S	e	c		3	0	S	e	c		M	o	r	e		B	a	c	k

STEP 10: Press the arrowhead beneath the word "More". The display should now look like:



STEP 11: Press the arrowhead beneath "2Min" (or select whatever time constant is most appropriate for your measurement conditions). The screen will briefly show the new value that has been set. The new value is immediately saved into sensor memory, and will be recalled even if you switch the instrument off and on again. The screen then returns to the 2nd Configuration menu (as shown above in Step 2).

STEP 12: Press the arrowhead beneath the word "More". Pressing the arrows beneath the words "Range" or "Disp" would lead you into sub-menus that would allow you to set parameters associated with the 4-20 mA analog outputs available on the rear of the Base Unit, or the display brightness, respectively. The analog output provides a signal that is proportional to the signal displayed on the front of the Base Unit (when not in the Configuration menus). The correspondence between the analog output and the signal value is determined by the range setting, available in these sub-menus.

STEP 13: Press the arrowhead beneath the word "More". Pressing the arrowhead beneath the words "Pswd" would lead you into a sub-menu that allows you to password-restrict access to the keypad functions. Pressing the arrowhead beneath the word "Reset" would allow you to reset all parameters to their factory default values.

STEP 14: Press the arrowhead beneath the word "Exit". This will exit you out of the configuration menus and return you to the "normal" display window. Note that communication via the USB or RS-232 ports is disabled while the configuration menu is active, so make sure to exit the configuration windows before trying to run the BE2100 User Interface software.

Now that you have a basic familiarity with the operation of the keypad interface, you may want to experiment with other features. Alternatively, a detailed step-by-step description of the keypad features is available in Appendix II. Once you have selected settings that are best for your application, you may wish to prevent accidental changes to these settings by turning on the password protection feature.

Verification of Sensor Performance

Before using the BE2100 sensor to measure biomass in a liquid culture you may wish to verify that it is performing as expected. This step is recommended when you are unpacking and using the instrument for the first time, but may also be used to periodically check sensor functionality. In addition to providing a verification of performance, the option of recalibrating the sensor is also provided.

The following procedure describes how to run the "Sensor Check" function via the keypad interface or the Virtual Instrument Software. If you plan to operate the instrument solely via the Virtual Instrument Software, you may wish to return this section later after you have installed the software (described in Part II of this manual).

Note that each BE2100 sensor is provided with a pair of "low" and "high" calibration cups with unique serial numbers. Due to small variations between the calibration cups, the same calibration cups should always be used with the sensor with which they were provided.

STEP 1: Check that the front face of the sensor is clean and free of fingerprints. The sensor face may be cleaned with commercial window cleaner, ethanol, or isopropanol using lint-free tissue. DO NOT USE ACETONE, as it may cause irreparable damage to components used in the sensor. Likewise, check and if necessary clean the surface of the "Low" and "High" calibration cups that come in contact with the front face of the sensor. **If you have more than one BE2100 sensor, note that the calibration cups provided for testing sensor performance are not identical and should be matched with the corresponding sensor.** The last 2 digits of the serial numbers printed on the sensor and low and high calibration cups should all match each other.

STEP 2: Remove all of the magnetic adapter sleeves from the sensor. Place the "Low" calibration cup over the front of the sensor. Make sure that the arrow marked on the calibration cup is pointing towards the arrow marked on the sensor. Connect the buckle components attached to opposite ends of the strap. Cinch the sensor against the calibration cup by pulling on the free end of the strap that protrudes from the buckle. Next close the latch on the back of the sensor --- this step should provide the final pressure needed to firmly seat the sensor against the calibration cup.

STEP 3: Start the "Sensor Check" function.

A. VIA THE KEYPAD INTERFACE (ON BE2100 OR BE2400 BASE UNITS)

Step i: Press any of the 4 arrowhead keys on the front of the Base Unit.

Note 1: If the Virtual Instrument software is currently communicating with the base unit (as indicated by "Remote Operation" on the top line of the display), keypad access is locked out. You will either need to suspend communication, or perform the "sensor check" from within the Virtual Instrument software (described below under "B").

Note 2: If you are not the 1st user of the instrument and a prior user has turned on password protection, before you can reach the above configuration menu you will be prompted to enter a password. You may wish to consult the prior user to obtain the password. Alternatively, if the password has been lost or forgotten, the password may be reset via the user interface software. If this is the case, skip this section for now, and come back to it later after you have installed the user interface software and reset the keypad password.

Step iia (BE2400 base units only): Press the arrowhead beneath the word "More". The top line of the display should now read "Select sensor number".

Step iib (BE2400 base units only): Select the sensor number for which you want to run the Sensor Check function.

Step iic: Press the arrowhead beneath the word "More".

Step iii: Press the arrowhead below "Chk". The top line of the display should now read "Sensor Check Mode:".
Step iv: Press the arrowhead below the word "Run". The top line of the display should now read "Attach Low Cal Cup".
Step v: Once the low calibration cup has been securely attached to the sensor (see step 2, above), press the arrowhead below the word "Ready".
Step vi: The instrument will make 25 measurements (lasting about 1 second each) on the low calibration cup. The standard deviation of the 25 measurements is used to assess stability. If the measurements were unstable, the message "Low Cup unstable RPT?" will be displayed. If this occurs, make sure that the front faces of the sensor and calibration cup are clean and dry and that the calibration cup is securely attached to sensor (see step 2, above). You will not be allowed to proceed to measurements with the high calibration cup until stable measurements on the low calibration cup have been collected.
Step vii: Once stable measurements on the low calibration cup have been collected, the message "Continue to Hi Cal" will be displayed. Press the arrowhead beneath the word "Yes". The top line of the display should now read "Attach High Cal Cup".
Step viii: Replace the low calibration cup with the high calibration cup. Make sure the cup is clean and securely attached to the sensor, in the same manner as for the low calibration cup (see step 2, above). Press the arrowhead beneath the word "Ready".
Step ix: 25 readings will now be collected on the high calibration cup. If the readings were unstable, the message "Hi Cup unstable RPT?" will be displayed. If this occurs, make sure that the front faces of the sensor and calibration cup are clean and dry and that the calibration cup is securely attached to sensor (see step 2, above). It will be necessary to repeat the low calibration cup (step v, above) measurement before proceeding again with the high calibration cup measurement. If the readings were stable, a "Pass/Fail" assessment of sensor performance will be made. This Pass / Fail assessment is based on a comparison of the present

measurements to prior measurements made on the calibration cups during manufacture.

B. VIA THE VIRTUAL INSTRUMENT SOFTWARE

Step 0: If you have not done so already, follow the steps under "Setting Up" of the BE2x00 Virtual Instrument Software (section II of this manual).

Step i: Start up the software and wait for the initial Device Search to complete. In the lower half of the screen, select the tab of the sensor for which you wish to run the Sensor Check function. Press the "Check Sensor" button (in the lower left of the screen).

Note: The Sensor Check function cannot be run while data acquisition is active.

Step ii: Once the low calibration cup has been securely attached to the sensor (see step 2, above), press the button labeled "Start Sensor Check", and then press "Start Low Cal Cup Meas".

Step iii: The instrument will make 25 measurements (lasting about 1 second each) on the low calibration cup. The standard deviation of the 25 measurements is used to assess stability. If the measurements were unstable, the message "The Low Cal Cup measurement was unstable." will be displayed. If this occurs, make sure that the front faces of the sensor and calibration cup are clean and dry and that the calibration cup is securely attached to sensor (see step 2, above). You will not be allowed to proceed to measurements with the high calibration cup measurements until stable measurements on the low calibration cup have been collected.

Step iv: Once stable measurements on the low calibration cup have been collected, the control button will read "Start High Cal Cup Meas". Replace the low calibration cup with the high calibration cup. Make sure the cup is clean and securely attached to the sensor, in the same manner as for the low calibration cup (see step 2, above). Press the button labeled "Start High Cal Cup Measurement", then the "OK" button in the pop-up screen that appears.

Step v: 25 readings will now be collected on the high calibration cup. If the readings were unstable, the message "The High Cal Cup measurement was unstable" will be displayed. If this occurs, make

sure that the front faces of the sensor and calibration cup are clean and dry and that the calibration cup is securely attached to sensor (see step 2, above). It will be necessary to repeat the low calibration cup (step ii, above) measurement before proceeding again with the high calibration cup measurement. If the readings were stable, a "Pass/Fail" assessment of sensor performance will be made. This Pass / Fail assessment is based on a comparison of the present measurements to prior measurements made on the calibration cups during manufacture.

STEP 4: Whether Pass or Fail is indicated at the end of the Calibration Cup measurements, you will be given the option of updating the sensor calibration coefficients based on the measurements just completed. If the "update" (or "Set New Coeff" in the Virtual Instrument software) option is selected, new sensor coefficients will be written into sensor memory. The new coefficients are determined by linearizing the newly completed calibration cup measurements to the original calibration cup measurements collected at the time of sensor manufacture. These new sensor coefficients will persist even across power cycling of the instrument. However, it is important to realize that selecting the "Reset" function (either via the keypad or the Virtual Instrument software), will reset sensor coefficients to their original factory settings.

SETTING UP ON A BIOREACTOR

Attaching the Sensor Head

STEP 1: Position the Optical Sensor Head on your bioreactor so that it will get a "good view" of the liquid culture. Most bioreactors are quite crowded with impellers, sparge tubes, pH probes, etc. This effect is exacerbated in very small bioreactors. Try to find a location relatively free of internal obstructions that would block or reflect emitted sensor light inside of the bioreactor. This effect is most important at low biomass levels. Your BE2100 sensor will achieve highest immunity to internal obstructions at highest biomass levels, near harvest time. The BE2100 sensor has maximum sensitivity to objects at a distance of about 2 cm from the front face of the sensor. However, at very low biomass, reflectance from farther objects can sometimes influence the readings.

STEP 2: Although your BE2100 Optical Sensor Head will adapt to virtually any diameter bioreactor, including flat glass viewing ports, because of the myriad bioreactor styles that the BE2100 may encounter, this step sometimes requires a "dry run". When mounting to all-glass cylindrical vessels, select and apply a combination of magnetic sensor adapters so that, when the front face of the sensor is depressed, the sensor front face can be made flush with the adapters. The best combination of adapters for this application is generally the 2 longest adapters (15 and 20 mm) stacked on top of each other. When mounting to flat recessed ports with a narrow aperture, it may necessary to use a different combination of adapters (or none). In such cases, the adapters should be selected so that the sensor front face is in intimate contact with the port/window, while the adapter(s) provides a stable mounting surface on the window flange. Note that alternate (and custom) screw-mounting options (as opposed to the strap mounting method described here) are also available for some bioreactor types. Inquire at BugLab (info@buglab.com) for more details. Pass the black mounting straps around the bioreactor. If the bioreactor has external metal rods, thread the straps under them if possible so that the straps are only in contact with the body of the bioreactor. Avoid twists in the straps --- the strap should lie flat around the perimeter of the bioreactor. Close the loop around the bioreactor by connecting the two buckle components attached to opposite ends of the strap (note: if the strap is not long enough, strap extenders are available). Cinch the sensor against the bioreactor by pulling on the free end of the strap that protrudes from the buckle. Next close the latch on the back of the sensor --- this step should provide the final pressure needed to firmly seat the sensor against the bioreactor. Do not use pliers or any gripping tools that would exert excessive force. The goal is to snug the sensor down on its black flexible gasket once the latch is closed so that the sensor is as close to the surface of the bioreactor as possible. If, by pushing on the back of the sensor, you can bring it closer to the bioreactor, the strap tension is insufficient. In this case, open the latch on the back of the sensor, cinch up the free strap end, and close the latch again. We strongly discourage the use of tapes or adhesives of any kind as they tend to foul the optical surfaces.

Please contact BugLab at (925) 208-1952 or info@buglab.com for further advice if you are having trouble.

STEP 3: Inspect the attached sensor to be sure that it is firmly and securely attached to the bioreactor. Readjust the mounting strap as required to achieve a firm fit.

Note: a few extra minutes adjusting the sensor for excellent fit will ensure especially reproducible results.

STEP 4 (OPTIONAL STEP RECOMMENDED FOR CROWDED BIOREACTORS): Temporarily set the sensor averaging time constant to 0, and observe the reported sensor reading. For sensors connected via a BE2100 or BE2400 base unit, this can be performed in stand-alone mode as already described. For sensors connected via a USB adapter the BE2x00 Virtual Instrument Software is required for configuration of and data acquisition from the sensor. In this case, install the software, set the time constant, and begin data acquisition following the instructions for operating the Virtual Instrument Software provided in Section II of this manual. Re-adjust the position of the sensor on the bioreactor to minimize the reported sensor reading. Ideally, on a bioreactor that is empty or that contains water or transparent media, a sensor reading of less than 0.1 Bug Units is desired, but this is not always possible in small (e.g. 1L and less) and crowded bioreactors. Once the best sensor location has been located and the sensor is tightly secured, return the sensor averaging time constant to its normal setting (the recommended setting for most applications is 2 minutes).

Important Reminders:

Do not expose the sensor to high temperatures.

Remove the sensor during any type of bioreactor autoclave cycle.

Operation

Your BE2100 sensor operates in the near infrared portion of the optical spectrum (at 850 nm) and is therefore invisible (or only slightly visible) to the human eye. You will not readily observe light emanating from the sensor. Normal operation is determined from the display on the Base Unit or the Virtual Instrument software.

When operating a BE2100 sensor connected through a BE2100 or BE2400 base unit, changing the configuration settings and observation of the biomass readings can be performed either in stand-alone mode with the keypad and display provided on the base unit, or through the Virtual Instrument software, when the base unit is connected to a personal computer. Sensors connected through a BE|USB adapter are operated solely via the Virtual Instrument software.

The BE2100 sensor results are reported in raw "Bug Units", baseline-corrected Bug Units, or User Calibrated units. "Bug Units" are generated at the time of sensor manufacture, by linearly scaling the measured optical (850 nm) reflectance from a calibration solution of *Saccharomyces cerevisiae* in a 14L glass fermentor to Optical Density (OD) measured at 600 nm in a 1 cm cuvette in a spectrophotometer (Perkin-Elmer, model Lambda 9), with appropriate dilution (Absorbance <0.5), and scaling by the dilution factor. Due to the differences between the OD measured between different commercially available spectrophotometers and the cell-size dependence of the correlation between biomass and optical scattering (see the "Theory of Operation" section for further details), when an absolute reading of biomass is needed, it is recommended to calibrate the BE2100 sensor to the particular organism you are growing using your preferred reference biomass method (e.g. dry cell weight, OD, etc). The high linearity of the BE2100 sensor makes performing such calibrations quite simple (see "User Calibration"). However, in many applications, where only measurements of the growth rate, or growth trend are needed, it is unnecessary to calibrate the BE2100 sensor, and measurements in "Bug Units" will suffice.

As with spectrophotometry, correction for the baseline scattering by the media alone is often useful in distinguishing the biomass from the media. As described in the following section, a baseline-correction function for the BE2100 sensor is provided for this purpose. Especially when working with complex media that may vary in turbidity from batch-to-batch, baseline correction on the media alone is recommended. When a User Calibration is used to transform the Bug Units data into some other reference, the transform is applied to the baseline-corrected result. For this reason, it is particularly important to consistently apply the baseline correction when applying User Calibrations.

The BE2100 can be sensitive to changes in sparge and stir rate, particularly at low OD's. If possible, establish ideal sparge and stir rates and leave them constant throughout the monitored fermentation. If changes are required in sparge or stir rate, note them. The BE2100 baseline may change as sparge or stir rate are changed, but it will still precisely track growth.

The effect of glass wall thickness between different types of bioreactor vessels has been tested and has a nearly negligible effect on BE2100 sensor response in the range of 6 to 13 mm (this range encompasses most glass vessels and well as flat glass ports on stainless steel reactors). The sensor incorporates a patented automatic mechanism to maintain a constant distance between the optical components and the outside surface of the bioreactor. However, this mechanism only functions if the sensor is held snugly to the bioreactor. For this reason, it is critical to ensure that the strap is tightly secured (as detailed in "Setting Up On a Bioreactor").

Baseline Correction

Baseline correction provides a means of subtracting off signals emanating from reflectance sources in the bioreactor that are not of interest. For example, in a typical application, the baseline will be measured just prior to inoculation, thereby subtracting off the contribution of media constituents from the reported "Bug Units". This function is similar to "zeroing" of a spectrophotometer using only medium prior to performing an OD measurement.

Before collecting a new baseline it is important to establish that the signal is stable. Viewing the signal in graphical format, such as provided in the Virtual Instrument software can be helpful for this purpose. If the baseline appears to be wandering excessively, it can sometimes be helpful to temporarily reduce the sensor averaging time constant to "0" while adjusting the sensor position and/or the bioreactor conditions (see Step 4 of "Setting Up for a Bioreactor Run").

The baseline correction function can be accessed both via the keypad interface on BE2100 or BE2400 Base Units or via the Virtual Instrument Software. Use of the keypad interface is described here. For the Virtual Instrument Software refer to section II.E.2 of this manual. In the following it is assumed that the sensor has already been attached to the bioreactor, and that a stable reading has been achieved.

STEP 0: Press any of the 4 arrowhead keys on the front of the Base Unit. [If you are using a BE2400 base unit, press the "More" button, then select the sensor number for which you want to set the baseline.] At this point,

the bottom line of the display should read “Base”, “Cal”, “More”, or “Exit”.

> **Note 1:** If the Virtual Instrument software is currently communicating with the BE2100 base unit (as indicated by “Remote Operation” on the top line of the display), keypad access is locked out. You will either need to suspend communication, or perform the baseline correction from within the Virtual Instrument software (section II.E.2).
>
> **Note 2:** If you are not the 1st user of the instrument and a prior user has turned on password protection, before you can reach the above configuration menu you will be prompted to enter a password. You may wish to consult the prior user to obtain the password. Alternatively, if the password has been lost or forgotten, the password may be reset via the user interface software. If this is the case, skip this section for now, and come back to it later after you have installed the user interface software and reset the keypad password.

STEP 1: Press the “Base” button.

STEP 2: Select the “Start” button and wait at least a few seconds before selecting the “Stop” button. Between the time that the Start and Stop buttons are pressed, the sensor readings will be averaged. The averaged values will be used to determine the new baseline setting. After the Stop button has been pressed, the new baseline is briefly displayed on the screen. The new value can be viewed again by pressing the “Show” button.

STEP 3: If the lower left of the screen shows “Off”, press the first arrowhead key so that the display changes to “On”. Even though the new baseline has been set, it will not be applied to the displayed data unless the correction is “On”.

STEP 4: Press the last arrowhead key twice to exit out of the configuration menus. The value now displayed on the screen is baseline-corrected and will continue to be until baseline correction is turned Off. A letter “C” displayed at the end of the 2nd display line indicates that baseline correction is currently being applied.

Note : Both the baseline value and the On/Off state will be remembered by the instrument across power cycles. However, selecting the “Reset” function, will turn baseline correction Off and reset the baseline value to zero.

WORKING WITH THE BE2100 and BE2400 BASE UNITS

Analog Output

Two analog current outputs are available on the rear panel of the BE2100 Base Unit. Both analog outputs produce a standard nominal range of 4 to 20 mA. The first analog output (AO1) is proportional to the signal being displayed on the front panel of the Base Unit. The second analog output (AO2) is proportional to rate of change of AO1 in log space. When AO1 is increasing exponentially, AO2 thus corresponds to the growth rate of AO1 (in units of inverse hours). The BE2400 Base Unit has 4 analog current outputs: one for each sensor. These analog outputs provide the same functionality as AO1 on the BE2100 Base Unit.

AO1 reflects whatever corrections are applied to the data being displayed on the base unit. So if baseline correction is on, then AO1 will also be baseline-corrected. Similarly, if user calibration is on, then AO1 will be in user-calibrated units. The growth rate calculation used to determine AO2 is independent of the units in which biomass is expressed. However, the baseline setting will affect this calculation, so particularly at low biomass it is critical that the baseline is set so that a baseline-corrected sensor reading of zero corresponds to zero biomass.

A signal of zero will always correspond to a nominal value of 4 mA on AO1, while a growth rate of zero will always correspond to a nominal value of 4 mA on AO2. The minimum signal that corresponds to a nominal value of 20 mA on analog output 1 will be determined by the "analog output range" variables (see key pad communication, Appendix II, section 1.v, for further details). Any signal higher than this minimum signal level will cause AO1 to output a nominal value of 20 mA. The minimum growth rate that corresponds to a nominal value of 20 mA on AO2 is determined by the range setting for AO2.

The rate of change reflected in AO2 is determined by a linear least squares fit to the natural logarithm of the data type currently being displayed on the front panel of the Base Unit vs. time (in hours). The "growth rate time window" variable determines the data set to be used for the slope calculation.

The range settings for AO1 and AO2 and the time window for AO2 can be set by 3 different methods: (1) the keypad interface on a BE2100 or BE2400 Base Unit, (2) the Virtual Instrument Software, and (3) by sending serial commands to the Base Unit. Further description of the keypad interface can be found in Appendix II of this manual. Part II of this manual describes how the Virtual Instrument Software can be used to

change the settings. Appendix III of this manual describes the serial commands ('R' and 'W') that can be used to programmatically set these parameters.

Note 1: The screw terminal for connecting the analog outputs is removable. It is supplied separately from the base unit and can be inserted by simply pushing it into the slot provided in the rear on the Base Unit. This feature makes it easier to connect wires to the screw terminals.

Note 2: The maximum resistance that the Base Unit can encounter to drive a full 20 mA is **500Ω.** By connecting a 500Ω resistor across the "+" and "-" terminals the analog output can be converted from a nominal 4-20 mA current source to a nominal 2-10V voltage source. Two 500Ω resistors are provided as accessories for this purpose.

Note 3: The 4 and 20 mA current levels are described above as "nominal" values because the actual values will vary slightly from instrument-to-instrument. For best accuracy when working with the analog outputs it is recommended to measure the actual currents (or voltages) produced when the signal is at 0 and when it is at or above the maximum determined by the range setting. An example calibration procedure is provided in Appendix IV of this manual.

Note 4: The digital-to-analog converters (DAC) in the base unit provide 12-bit precision (meaning that the minimum step size is ~4 μA). In order to ensure best performance it is important to match the Range setting to the maximum anticipated biomass reading.

Keypad Lockout

Many functions of the BE2x00 instrument may be accessed both by keypad interface on a Base Unit and programmatically through serial interface commands. In order to prevent conflict between these different methods of accessing the same functions, it is helpful to establish a prioritization between them. When the BE2x00 is being controlled remotely through the Virtual Instrument software, access to the keypad is restricted. For users writing their own interface code, it is recommended that access be similarly restricted (using the 'J' command, described in Appendix III).

When the Virtual Instrument software is running the message "Remote Operation" will be displayed on the top line of the BE2100 or BE2400 Base Unit display. In this state, the keypad configuration menus cannot be accessed. The Base Unit always starts up in Local Mode when powered on. While the keypad configuration menu is active, the keypad access will remain in Local Mode until the configuration menu has been exited

by the user. For this reason, you should **make sure to exit out of the configuration menus before attempting to start the Virtual Instrument software.**

Display Scrolling (BE2400 Base Units only)

When operated in local mode (without a computer) the BE2400 display can be set to scroll through all attached sensors, or to lock onto 1 particular sensor (see **Introduction to the BE2400 Base Unit Functions**). When operated in remote mode the scrolling mode is controlled through the Device Configuration table. The last column in the Device Configuration table controls which sensor are included ("On") or excluded ("Off") from display scrolling.

Password Locking and Unlocking

When operated in local mode the BE2100 and BE2400 base units have a password protection feature that can be turned on, to prevent inadvertent changing of configuration settings. Password protection can be turned On via the keypad by selecting the "Pswd" option, Setting a 6 digit password ("Set"), and then turning password protection On. Once the configuration menu has been exited, subsequent entry into the configuration menus will first require password entry.

The BE2x00 Virtual Instrument software provides a means of Unlocking the password restriction, if the password has be lost or forgotten. This accomplished within the Device Configuration table by selecting the table cells in the column labeled "Base Unit Password Lock Status". Four options are available:

Unlock(ed): Turns Off password protection.
Lock(ed): Turns On password protection.
Reset and Unlock: Turns Off password protection and resets the password to "111111".
Reset and Lock: Turns On password protection and resets the password to "111111".

Warning Messages

It is possible to overwhelm the BE2100 sensor if ambient light conditions are extremely high. You may see the warning shown below occasionally. If it is infrequent, it may be safely ignored. The BE2100 always becomes vastly more resistant to high ambient light as your organism reaches higher optical density. If the sensor is exposed to direct sunlight, halogen

lamps at close range, or similar conditions, the following warning condition may be posted:

The signal displayed is accurate, but the user is encouraged to lower ambient lighting conditions.

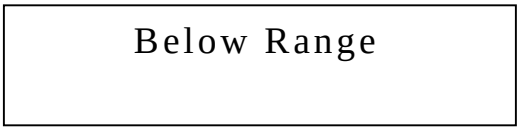
If User Calibration is turned on, but the present measurement is outside of the calibration range, the following warning is displayed:

Error Messages

If the ambient light conditions are so high as to prevent accurate measurements, the BE2100 will post the following error message:

You must lower ambient lighting conditions in order to measure accurately.

If the sensor is operated during conditions where the OD of the fermentation is below the range of the internal “Bug Units” calibration (less than 0.001 Bug Units), the BE2100 will post the following error message:



This error is automatically resolved when the OD of the fermentation reaches the lowest OD of the calibration.

If the sensor is operated during conditions where the OD of the fermentation is above the range of the internal “Bug Units” calibration (greater than 1000 Bug Units), the BE2100 will post the following error message:

Above Range

The Base Unit will inform you if the sensor has become disconnected:

Sensor Disconnected

PRINCIPLES OF OPERATION

The BE2100 sensor employs a near infrared (850 nm) laser to non-invasively measure back-scattering from biomass within liquid cultures. The laser is directed through the wall of transparent (glass or plastic) vessels or through a glass port window of stainless steel bioreactors. When the laser light is scattered by the cells or microorganisms, it creates a "glow ball" of monochromatic light. The intensity and size of the glow ball is dependent on the biomass within the liquid culture. At early stages of growth, when the biomass is low, the glow ball will be large in size and weak in intensity. As the cells or microorganisms grow and divide, the density will increase and the glow ball will reduce in size and increase in intensity. The detectors within the BE2100 sensor are sensitive to the intensity of light back-scattered from within the glow balls. Further, the photo-sensor is arranged such that there are multiple separation distances between the detectors and light sources. This is of critical importance to the BE2100 technique. The sensor firmware automatically arbitrates among the source-detector pairs, selecting that pair with the most linear response to biomass at the particular biomass currently being measured.[1] By combining the signals from the multiple detectors due to the multiple light sources, the biomass in the liquid culture is determined with a dynamic range that is orders of magnitude larger than immersion transmission techniques.[1] Although classical Optical Density (OD) measurements in a spectrophotometer require dilution in order to accurately determine biomass greater than about 0.5 OD(600 nm), the BE2100 sensor is able to determine biomass from 0.1 to 300 OD(600 nm), without dilution, or any liquid handling. The high linearity of the BE2100 sensor allows growth rate to be accurately and rapidly assessed, whether or not the sensor has been user-calibrated biomass over a very wide range of response.

One of the advantages of using an infrared laser source for measuring biomass is the avoidance of light absorbance by colored media components (and colored vessel materials). This allows for the measurement of true scattering rather than a combination of absorbance and scattering. As a result, a highly linear relationship is maintained between biomass concentration and the measured optical reflectance. However, it is also important to realize that for biomass containing strongly visible-light-absorbing chromophores (e.g. photosynthetic algae), the chromophore absorbance may affect the agreement between a conventional OD measurement and the result reported by the BE2100 sensor. The OD measured in the visible range by a spectrophotometer will be influenced by both chromophore absorbance *and* cell scattering, whereas the OD reported by the BE2100 sensor will be based only on cell scattering. In such situations, if the relationship between chromophore concentration and biomass is relatively fixed, it still may be possible to

generate a strong correlation between OD measured by conventional methods and that reported by the BE2100 sensor. However, it is important to be aware that changes in chromophore concentration that are not accompanied by biomass change would not affect the result reported by the BE2100 sensor, but would skew the results determined by conventional visible spectrophotometry.

Another important consideration when comparing BE2100 sensor results with conventional spectrophotometry, is the optical design of the spectrophotometer. Most spectrophotometers are designed to make accurate measurements of absorbance, *but not scattering*. A determination of chromophore absorbance requires only a comparison of how much light is extinguished within a sample when the chromophore is present at different concentration levels (e.g. zero and a known concentration). By contrast, in a scattering measurement, light is deviated from its path, rather than being extinguished. As a result, the measured amount of scattering will be dependent on the area and angle of scattered light that is captured by the detection system. Since the detector size and geometric arrangement is not standardized between different commercial spectrophotometers, the Optical Density determined for biomass samples can vary significantly (e.g. 50% or more variation between different spectrophotometer models!). For this reason, if you would like the BE2100 sensor to report results in OD units, it will be necessary to calibrate to the specific spectrophotometer that is used for the off-line OD measurement. A simple step-by-step guide for generating a custom calibration for the BE2100 Sensor is described below (***User Calibration***, Section II.G).

The relationship between back-scattered light intensity (as measured by the BE2100) and biomass (such as dry cell weight) is weakly dependent on the size of the scattering particles. For this reason, it is recommended that separate calibrations be used for organisms with grossly different cell sizes, such as *Escherichia coli* (typical cell diameter 0.5-1 μm) and *Saccharomyces cerevisiae* (typical cell diameter: 5-10 μm). For mono-disperse cell cultures, these cell size differences can be compensated using a single multiplicative factor ("calibration slope"). Note that the 10-fold difference in cell diameters of these 2 microorganisms only has about a 2-fold effect on the BE2100 calibration to biomass. For this reason, minor variations in cell diameter, such as are observed in different stages of growth, or between different strains of the same organism, will have a relatively minor effect on the BE2100 sensor accuracy. Note that correlation between biomass and OD measurements performed using conventional spectrophotometry are also cell-size dependent, and that this cell-size dependence is somewhat different than for the BE2100 sensor, due to the difference between the optical measurement geometries (e.g. transmission vs. reflectance).

More serious consideration must be given to organisms that do not grow as mono-disperse cells (e.g. filamentous growth). The relationship between OD (whether determined by conventional means or the BE2100 sensor) and biomass is non-linear for organisms that are not mono-disperse in a liquid culture. As a result, OD can only be expected to provide an accurate measure of biomass for mono-disperse liquid cultures. For organisms that grow in clusters, OD can only be expected to provide a qualitative estimate of biomass.

Correlation between biomass and BE2100 sensor measurements will generally be highest during the exponential (aka "logarithmic") phase of cell growth. Cell lysis results in a dramatic change in the average particle size. Once significant cell lysis has begun, such as typically occurs during the stationary phase of cultures, there will no longer be a linear relationship between biomass and optical measurements of scattering (by either conventional spectrophotometry or by the BE2100 sensor). Attempts to apply a non-linear fit to accommodate more than one particle size at a time (such as happens due to cell lysis) is not likely to be reliable because there is insufficient information to distinguish between changes in particle size vs. changes in number of particles. For this reason, when generating new calibrations, we recommend only using data collected prior to the transition between logarithmic and stationary phases of cell growth.

What about the effects on measurement accuracy of vessel size, shape, wall thickness, and material? The BE2100 sensor instrument is designed to provide accurate results over a wide range of vessel types. This is accomplished by limiting the optical measurement volume and restricting the allowed geometry between the vessel and instrument.[1,2] Through a combination of these methods, the BE2100 sensor has been demonstrated to provide high accuracy (<15%) over a wide range of vessel types and wall thicknesses (up to 13 mm).[1]

References

1. U.S. Patent 8,603,772. "Particle Sensor with Wide Linear Range".

2. U.S. Patent 7,100,462. "Self-Adjusting Sensor Mounting Device".

BE2x00 Software

Minimum System requirements:

1. Windows XP / Vista / 7 / 8 / 8.1 (32 or 64 bit) Operating System
2. Minimum of 256 MB of RAM
3. Minimum 200 MB free hard disk space.
4. 1024 by 768 resolution (or higher) video adapter.
5. Microsoft-compatible mouse.
6. Available 9-pin RS-232 or USB communications port.

The BE2x00 software runs in the LabVIEW™ operating environment. Two separate programs are provided: (1) the "BE2x00 Virtual Instrument", and (2) the "BE2x00 Data Viewer". The Virtual Instrument software gives you the ability to chart the progress of your fermentation in real time, and annotate important events. Most importantly, the software allows you to calibrate your BE2100 sensor to the units of your choice. This calibration can then be written into sensor memory, allowing the BE2x00 instrument to run in calibrated mode without being connected to a computer. The Data Viewer software allows you to open, view, manipulate, and re-save data files that were previously acquired with the Virtual Instrument software. The Data Viewer software does not communicate with BE2x00 instruments and can be run at the same time as the Virtual Instrument software.

Conventions and Shortcuts

1. **Bold** text is used to indicate menu items and buttons that you may select with your mouse, key combinations that you may execute on your keyboard, and names of control and indicators on the graphical user interface.

2. *Italic* text is used to indicate window names.

3. ***Bold italic*** text is used to indicate sections of this manual.

4. The » sign is used to indicate sub-levels of menu commands. For example:

   Start » Settings » Control Panel » Add/Remove Programs

   means; select the **Start** menu, then select the **Settings** sub-menu, further select the **Control Panel** sub-menu, and then finally select the **Add/Remove Programs** sub-menu.

Note: Before plugging a BE2x00 device into your computer, make sure you have installed the software first!

STEP 1: Insert the BE2x00 software Compact Disk (CD) into your computer.

STEP 2: If a Windows *AutoPlay* message pops up, under "Install or run program from your media" choose "Run setup.exe". If the installation does not start automatically, browse the CD for the "setup.exe" file and double-click on it. If Windows asks "Do you want to allow the following program (setup.exe)…to make changes to this computer", click on the **Yes** button.

STEP 3: You will be prompted to choose destination directories for both the BE2x00 (BugLab) software and the LabView (National Instruments) run-time engine used by the BE2x00 software. Click on the browse buttons if you want to install these programs somewhere other than the default directories shown. Otherwise, click on the **Next** button.

STEP 4: Review the software license agreements. If you agree, then click on the "**I accept…**" buttons and then the **Next** button.

STEP 5: A summary of the software components that are about to be installed is next displayed. Click on the **Next** button to begin installation. Installation may take several minutes.

STEP 6: If the software has successfully been installed you should see an *Installation Complete* window. Clicking on the **Next** button.

STEP 7: The driver for the USB virtual communication port device in the OD Scanner instrument is next installed. If a windows pop-up message asks whether you want to allow changes to your computer, select "Yes". A window entitled "FTDI CDM drivers" should open. Select "Extract". Once extraction is complete, the "Device Driver Installation Wizard" should automatically start. Select "Next" and wait for a confirmation that the driver was successfully installed. Select "Finish" to exit out of the Installation Wizard.

STEP 1: Configure the power settings on your computer so that sleep mode is disabled.

Step i: Select **Start » Control Panel** (in Windows 8, from the desktop, simultaneously press the Windows and the "C" key, and select "Settings", and then "Control Panel").

Step ii: Select **Hardware and Sound » Power Options » Edit Plan Settings**.

Step iii: Choose "Never" for the Sleep setting.

Step iv: Press the "Save Changes" button, and then exit out of the Control Panel window.

Note: Serial communication is interrupted when a windows computer goes into "sleep" mode. This will cause the *BE2x00 Virtual Instrument* software to lose it's connection with the sensor.

STEP 2: If you are working with a BE2100 Base Unit, select the communication port type on the base unit, as follows.

Step i: Press any of the 4 arrowhead keys on the front of the Base Unit.

Step ii: Press the arrowhead beneath the word "More" three times. The top line of the display should now read "Configuration Menu 4".

Step iii: Press the arrowhead below "Comm". The top line of the display should now read "Communication Select".

Step iv: Press the arrowhead below "USB" or "RS232" depending on the type of communication port you are using. Your new selection (along with the default value) will be displayed briefly on the screen.

Step v: Press the arrowhead beneath the word "Exit".

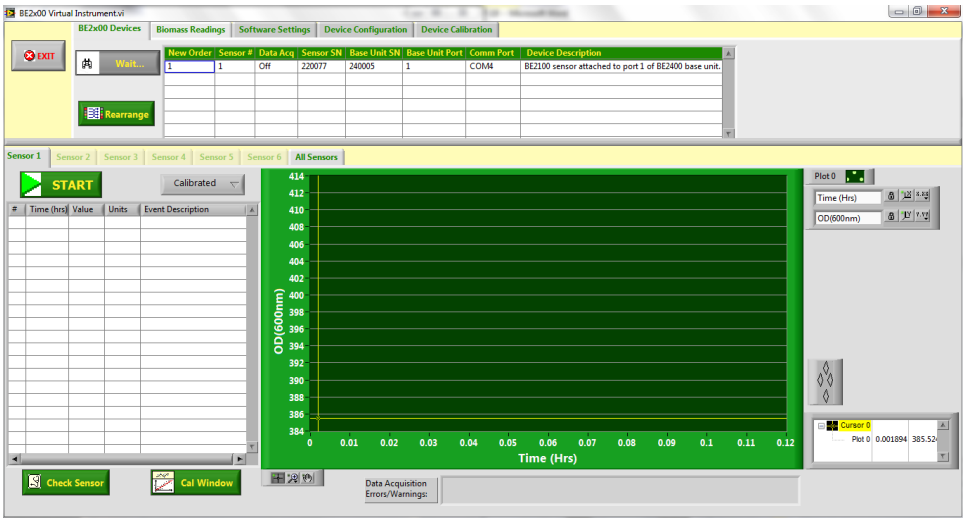
Note 1: Virtual instrument communication with the base unit is "locked out" while the configuration menus are active. Make sure you have returned to the "normal" screen before proceeding to the next step.

Note 2: When "USB" is selected at the communication type, RS232 communication is still functional, as long as a USB cable is not also connected. However, when "RS232" is selected as the communication type, USB communication is disabled.

Note 3: If you working with a BE2400 Base Unit you will notice that there is no "Comm" setting. On BE2400 Base Units USB communication is automatically active

whenever USB power is sensed. When communicating via RS-232, make sure that the USB cable is unplugged.

STEP 2: Connect the RS-232 or USB cable between your Base Unit (or BE|USB Adapter) and your computer. If this is the first time you are connecting the instrument to the computer via USB you should see a Windows "Installing Device Driver Software" message pop up. If so, wait until a second message pops up saying "Your device is ready to use" before proceeding to the next step.



BE2x00 Virtual Instrument software main window.

STEP 3: Launch the BE2x00 software:

In Windows 7 and earlier: select **Start » All Programs » BugLab » BE2x00 » BE2x00 Virtual Instrument.exe**.

In Windows 8: Go to the start screen, right-click over an empty space, and then select "All apps" (lower right-hand corner of the screen). Scroll through the applications until you find "**BE2x00 Virtual Instrument"**. Click once on the application.

STEP 4: When the program is started up, a search is automatically initiated for all connected BE2x00 devices. For those BE2x00 devices that are identified, all configuration settings are read from instrument memory and put into program memory. This process may take several minutes, but once complete, a list of all available devices will be shown in the "BE2x00 Devices" page at the top of the screen. All configuration settings, as read from instrument memory, can also be viewed in the "Device Configuration" and "Device Calibration" pages.

The bar separating the top and bottom portions of the window can be adjusted (over a limited range) by clicking and holding the mouse on the bar and moving the bar to the new desired position before releasing the mouse again. Notice that Sensor Tabs at the bottom of the page are enabled according to the number of BE2100 sensors that were identified. The sensor number on the sensor tabs corresponds to the "sensor #" columns in the configuration tables at the top of the page. If you wish to change the order in which the sensors are numbered you can do so by entering the desired order into the "New Order" column of the "BE2x00 Devices" page and then selecting the "Rearrange" button. The sensor numbering will be automatically updated in all configuration tables and in the Sensor Tabs at the bottom of the page.

STEP 5: Select the "Software Settings" tab at the top of the page. The "Sampling Interval" column in the table determines the frequency with which data is read from the sensor, displayed in graphical form, and saved to file (data is always saved to file as soon as it is acquired). The default setting for the Sampling Interval is 60 seconds. If you wish to change the sampling interval, type in a new value (in units of seconds). Notice that the sampling interval is individually configurable for all available sensors.

STEP 6A: Select the "Device Configuration" tab at the top of the page. The table column labeled "**Ave. Time** (2 min)" determines the averaging time constant setting for the BE2100 sensor data. The averaging time constant determines how quickly the sensor responds to change. The larger the time constant the smoother the data and the slower the response. The default setting is 2 minutes. To set the averaging time to a new value, click on the current value and select from among the choices (ranging from 0 seconds to 8 minutes). Note that as soon as you select a new value, it is written into sensor memory. The new value will persist across power cycling, and will automatically be loaded into the table if you restart the program. The settings are individually configurable for each BE2100 sensor.

STEP 6B (OPTIONAL): The Device Configuration Table column labeled "**Growth Window** (8 min)" determines the time window over which the determination of growth rate is performed. The growth rate is determined from a linear fit to the natural logarithm of the biomass vs. time. During exponential growth the slope of

this fit corresponds to the growth rate of the organism (provided in units of inverse hours). The Growth Window setting determines the time window over which the fit is performed. If you plan to use this feature and want to change the Growth Window setting, click on the current setting and select from among the allowed settings (ranging from 1 to 32 minutes).

STEP 6C (OPTIONAL): The Device Configuration Table columns labeled "**Base Corr.** (Off)" and "**Base. Val.** (0.0)" determine respectively whether baseline correction is applied to the data and the value of this correction. Under normal operation it is recommended that the baseline correction be determined after initiating data collection (see the section "Setting the Baseline").

STEP 6D (OPTIONAL): The Device Configuration Table columns labeled "**User Cal.** (Off)" determines whether a custom user calibration is used to transform the sensor data into units other than standard "Bug Units". Step-by-step procedures for generating a new calibration are described later, in the ***Calibration*** section of this manual. If you have not already generated a custom calibration, set **User Cal.** to **Off** (if it is currently **On)**. If you have already generated a custom calibration and wish to now apply it to the new data you are about to collect, select the "Device Calibration" tab near the top of the screen and check that the calibration coefficients and units have been properly set. If the settings are not correct, follow the steps in the ***Calibration*** section of this manual. Once the calibration coefficients and units are properly set, return to the Device Configuration table and set **User Cal.** to **On**.

Initiating Data Collection

STEP 1: Select the Sensor Tab (lower part of the window) corresponding to the sensor number for which you want to begin data collection. Press the **Start** button.

STEP 2: You will be prompted to enter a file name for your data. A default filename is automatically created, which consists of the year, month, day, hour, minute, and second at which you pressed the Start button. Choose the directory where you wish to store the data and then modify the file name as desired and press **OK**. The **.bug* extension is added automatically. Data will automatically be

saved to this file as soon as it is transmitted from the BE2x00 device.

If you selected a file name that already exists you will have the options of choosing another file name, overwriting the existing file, or appending to the existing file. Note that selecting **Overwrite** will result in the old data file(s) being deleted, so choose this option carefully!

Selecting **Append** allows you to continue a previously aborted experiment. If you choose this option, the old data will be loaded and the new data will be added to it. The time expired between the earlier and the current experiment will be automatically accounted for. Appending is only allowed if the serial number of the currently selected BE2100 sensor matches that in the file header of the previously written data file. Also, all settings are read from the old data and event file and used to update the sensor configuration before the appended data acquisition commences.

Selecting **Change** will return you to the *Select a filename for data storage* Window.

STEP 3: At this point, you are now actively collecting data and it will begin to appear on the graph window for the selected sensor number. New data points will appear at the time interval you previously chose (see **Setting up and configuring**, step 5), so do not be alarmed if you do not immediately see new data points appearing on the graph. When data collection is started, the "Biomass Readings" table is automatically selected in the top portion of the program window. Each time a new reading is taken by the sensor this table is updated with the new time-stamp and biomass reading. The biomass reading displayed in this table is processed according to the configuration settings; if baseline correction is on, then this result will be baseline corrected; and if user calibration is on, the biomass will be reported in user calibrated units. A numerical Error Code and its interpretation are also displayed in the table. An error code of 0 and the error code interpretation "Normal Operation" are displayed when the sensor is operating in the normal range. If the sensor is operating outside of its normal range, warning or error messages will be displayed. See ***Part I, Working with the BE2100 and BE2400 Base Units*** in this manual for more details on the types of warning or error messages that may be displayed. The **Display Mode** selector to the upper left of the graph allows you to select the data that is displayed on

the graph (either **Raw, Baseline Corr., Calibrated** or **Growth Rate**).

Setting the Baseline

The Baseline setting is used to subtract a constant offset from the raw and/or calibrated sensor data. For example, when running in **Calibrated** mode using a calibration file that converts the raw Bug Units into OD units, setting the baseline is just like "zeroing" your spectrophotometer with a reference measurement. In a typical experiment, the baseline is set near the beginning of a fermentation run, just prior to inoculation, as described below.

To set the baseline, the user normally specifies a range (start and end point, as selected by right mouse button), and the baseline is determined by averaging over the specified range. Alternatively, the baseline can also be set manually. The following step-by-step description shows how to use either method.

STEP 1: While running an experiment, the Baseline can be set by positioning the mouse arrow over the graph window at the time position at which you would like to start baseline averaging and clicking on the right mouse button. From the drop-down lists that appear choose "Create Annotation" >> "Baseline Start".

STEP 2: The *Add Annotation* Window now becomes active. Under the box labeled "Positioning Method:" notice that the selected method is "Cursor". This means that the Baseline Start will be marked at the position at which you right-clicked the mouse. Alternatively, if you wish to start the baseline averaging at the most recently collected data point, select "Add to End". Click on the "Add Annotation" button at the bottom left of the pop-up window.

STEP 3: Notice that a red marker and number have been added onto the graph, indicating the start of baseline averaging. An "event" has also been added to the "Event List" located to the left of the graph. Continue the data acquisition process until you would like to define the end point for baseline determination. Position the mouse arrow over the graph window at the time position at which you would like to end baseline averaging and clicking on the right mouse button. From the drop-down list that appears select "Create Annotation" >> "Baseline End".

STEP 4: The *Add Annotation* Window again becomes active. Leave the "Positioning Method:" set to "Cursor Position" if wish

to mark the "Baseline End" at the point where the mouse was right-clicked. Alternatively, if you wish to start the baseline averaging at the most recently collected data point, select "Add to End". Click on the "Add Annotation" button at the bottom left of the pop-up window.

STEP 5: You have now defined the start and end points for baseline determination, so you are ready to set the baseline. Right click the mouse button anywhere over the graph. From the drop-down list that appears select "Create Annotation" >> "Baseline Set".

STEP 6: The *Set Baseline Interactively* Window now appears. When this window is brought up, the current baseline value is automatically read from the sensor and is displayed in the "Baseline Value" box. To define a new baseline based on the start and end points you have just selected, choose "Compute from graph" in the box labeled "Baseline Method". The newly computed value is displayed in the "Baseline Value" box. Alternatively, if you wished to set the baseline manually, you could have selected "Set manually" as the "Baseline Method". Then, you could manually enter a new value into the "Baseline Value" box. Note that when the "Set manually" option is selected, the current sensor reading is written into the Baseline Value window, but you may edit this value, if desired. Select the "OK" button at the bottom left of the pop-up window.

You have now set a new baseline value. The time at which the new baseline value was set is recorded and displayed in the text box in the main window. In addition, the annotation is numbered and marked on the graph. Notice that the baseline is applied only prospectively (data points that were acquired prior to setting the baseline are not affected by the new baseline).

When a new baseline is set via this "create annotations" method, the baseline correction is automatically turned On. Baseline correction can also be manually turned On or Off within the Device Configuration table. If you are using a BE2100 or BE2400 Base Unit the display will include a "C" at the end of the second line when baseline correction is On.

Modifying the Data Acquisition Window

Several options are available to allow you to customize the manner in which the data is displayed in the *Data Acquisition Window*.

1. You can change the scaling of the graph axes:

By right-clicking over the graph, the autoscaling of both the X and Y axes can be turned on or off. When auto-scaling is turned on the BE2x00 software will select axis limits that best display all of the data collected since the data acquisition was begun. When autoscaling is turned off, you can change the values on the axes by clicking one or more of the extreme values of the grid labels and changing their values. The pan (depicted as a hand) and zoom (depicted as a magnifying glass) features at the left bottom of the graph can also be used to change the range of the graph that is displayed. If you pan or zoom while autoscaling is on, the graph will rescale whenever new data is added to the graph that falls outside of the current window. Regardless of the mode selected, all of the data will always be stored to your selected data file. Note: autoscaling can also be turned on and off by clicking on the lock symbol within the scale legend.

2. You can use the cursor to read the value of a specific data point:

   First, select the cursor tool, which is depicted with cross-hairs and is located to the bottom left of the graph. Next, use the cursor positioning tool (four diamond shapes located at the bottom center of the graph) to move the cursor to the desired location. The cursor can also be moved with the mouse, by left-clicking the mouse over the cursor, and dragging the cursor to a new location whiling keeping the mouse button depressed. The X and Y values of the data point where the cursor is located are indicated within the cursor box (at the bottom, right of the graph).

   By right mouse-clicking over the cursor box, several other options can be accessed. The cursor can be centered within the screen by selecting the "Bring to Center" option. Additional cursors can also be created or deleted.

3. You can change the displayed data type by selecting the switch at the upper left of the graph to **Raw**, **Baseline Corr.**, **Calibrated**, **Growth Rate**, or **Error Code**. Note that the unless otherwise selected, the displayed data type will be set according to the Device Configuration table (if User Cal. is On, then the data type will be **Calibrated**; if User Cal. is Off and Baseline Corr. is On, then the data type will be **Baseline Corr.**; if both User. Cal. and Base. Corr. are Off, then the data type will be **Raw**). By selecting the **Growth Rate** data type you can observe a real-time estimate of the exponential growth rate of your organism (in units of inverse hours). By

selecting the **Error Code** data type, you can quickly identify whether any data points were collected under conditions of error (negative error codes), warning (positive error codes), or normal operation (error code = 0).

> **Note**: In all of the display views, data points that were collected under normal conditions are displayed in light green. Data points collected under warning or error conditions are depicted in yellow or red, respectively.

4. By right-clicking on the data markers within the plot legend (to the upper right of the graph) you can change many aspects of the graph including the plot style, marker color, and marker symbol.

Recording Events during Data Collection

A helpful tool provided in the data acquisition graph windows is annotation (or event marking). As you alter conditions during a bioreactor run, you can easily note them, and they will be automatically time-stamped.

Note: Data acquisition must be started before the create annotation feature becomes active in the individual sensor graphs.

STEP 1: To record an event, simply right-click on the area of the plot where you wish to record an annotation, and select "Create Annotation".

Several pre-defined event types are available, including:

- Inoculation
- Calibration Sample Removal
- Sparge Rate Change
- Agitation Rate Change
- Foam Breaker Rate Change
- pH Change
- Temperature Change
- Nutrient Addition
- Induction
- Harvesting
- Baseline Start
- Baseline End
- Baseline Set
- Delete All Annotations

You also can enter a **User-Defined** event for non-standard events.

STEP 2: Select one of the event labels from the list.

STEP 3: In the *Add Annotation* window, various options can be set and recorded depending on what event is selected. Additional comments can be added within the text box labeled "Edit the annotation text below, if desired". A "Value" and "Units" associated with the event can also be entered. For example, if the agitation rate was set to 500 rpm, 500 could be entered into the "Value" box and "rpm" could be entered into the "Units" box.

The positioning of the annotation on the graph is determined by the "Positioning Method" selector box. The annotation can be added to the end of the dataset (corresponding to the moment in time when "Add Annotation" was initiated), or it can be added at a manually selected point in time (time can be specified under the "Graph Position" label), or it can be added at the cursor position where the mouse was clicked.

STEP 4: When all parameters have been satisfactorily edited, click on the **Add Annotation** button. The annotation is numbered and marked on the graph, as well as being displayed in a text box to the left of the graph, for future reference.

Note: Like the sensor data, the event data is saved to file as soon as it is generated. The filename into which the event data is saved is the same as the sensor data file except the extension is "*.evt" instead of "*.bug".

Editing Annotations

Annotations can be edited by double-clicking on cells within the event table and typing in a new value or text string. Modifications made to the event table are saved to file as soon as you finish typing them in and hit the enter key, or exit the table cell you were editing.

Events may also be deleted by right-clicking the mouse over an annotation, and selecting "Delete Annotation". This action will result in removal of the annotation marker from the graph, deletion of the event from the event table, and deletion of the event from the saved event file.

Simultaneous Data Collection from Multiple Sensors

Following the same procedures described above, data acquisition can be initiated on up to 6 BE2100 sensors simultaneously. If a new BE2x00 device has been connected since the last search was performed, select the "Search Again" button within the BE2x00 Device window (top portion of the screen). Performing a new search will not interrupt data acquisition on devices that are already active.

Data from multiple sensors can be overlaid and viewed by selecting the "All Sensors" tab. The data type that is displayed for each sensor on the "All Sensors" graph is determined by the data type that was selected on the individual sensor plots. Thus, if the "Calibrated" data type was selected in the "Sensor 1" graph window, the data that will be displayed for Sensor 1 in the "All Sensors" window, will also be "Calibrated". The sensors that are displayed in the "All Sensors" graph can be controlled by using the "On/Off" column in the "Multi-Plot Settings" Table. The relative positioning of the different sensors can be editing the "X Offset", "Y Offset", and "Y Scaling" values in the "Multi-Plot Settings" Table.

Annotations can also be added to the "All Sensors" graph. However, these annotations are only saved in temporary memory and are not written to file. In order to save annotations into permanent record, they must be marked on the individual sensor graphs.

Terminating Data Collection

Selecting **STOP** terminates data collection.

USER CALIBRATION

If you wish to convert the BE2100 sensor data into reference units other than Bug Units, you will need to apply a calibration. Your BE2x00 software can help you to collect and then apply a custom calibration file. When applying your calibration file you will no longer need to perform aliquot extraction (or any other classical method) in order to determine biomass or related quantities, as the BE2100 sensor will now do that for you.

Collecting Calibration Data

This section describes how to collect a calibration file for your BE2100 sensor. Your calibration will provide the most accurate results if the positioning of the sensor on the bioreactor and the conditions inside the

bioreactor are similar between the calibration run and subsequent runs in which the calibration is applied.

STEP 1: Run a fermentation as you normally would with the BE2100 sensor properly attached and monitoring. Follow the directions in the earlier section of this manual entitled ***Initiating Data Collection***.

STEP 2: At representative points during the fermentation, collect calibration samples. Right-click over the graph each time a calibration sample is removed. You will note a special Annotation type called **Calibration Sample Removal**. Each time you record a **Calibration Sample Removal** Event, the software records the time, the event number, and the raw sensor output. Note that the off-line reference value and units can also be recorded now in the "Value" and "Units" fields. However, more commonly, the off-line values will be entered at some later time, as they become available.

Click on the "Add Annotation" button. Notice that calibration events are marked on the graph in blue. Use the event number to keep track of the samples you collect for off-line analysis.

STEP 3: Repeat step 2 until a full calibration set has been collected. The calibration file must contain a minimum of one point, but we strongly suggest more. It is recommended that calibration points spanning the lowest and highest biomass are recorded. Collection of calibration points during the exponential growth phase (prior to stationary phase) usually results in the most successful calibration.

STEP 4: Press **STOP** when a full calibration set has been collected.

Editing, Generating, and Saving a Calibration

STEP 1: Once you have completed a fermentation run during which you collected calibration samples, select **Cal Window** within the sensor graph window for which you want to generate a calibration. The *User Calibration* Window appears. The data table on the left side of the screen is automatically populated with the calibration samples from the Event List currently loaded. If you wish to read in the calibration samples from a different file, select "Read from Event File". Alternatively, if you wish to bring up a previously saved calibration file, select "Read from Cal File".

STEP 2: The data table contains four columns: "Event #", "Raw Sensor", "Baseline", and "Calibration". The "Sensor" column contains the data reported by the BE2100 sensor at the time at which the calibration sample was extracted. The "Baseline" column contains the baseline value that was used to collect the BE2100 sensor data. The "Calibration" column contains the matching off-line reference data that will be used to generate the calibration. If you have not entered any calibration data yet, the Calibration value will be -1. Add the off-line reference values to the Calibration column. Make sure that the "Event #" corresponds to the correct sample. The data displayed on the graph will be updated as soon as you enter it into the table.

STEP 3: If necessary, adjust the Raw Sensor and Calibration measurement offsets by using the Adjust Baseline, Fixed Intercept?, and Intercept controls. The value in the Baseline column of the calibration table is subtracted from the Raw Sensor readings before correlating them to the Calibration values. By pressing the Adjust Baseline button, you can simultaneously change the Baseline setting for all measurements in the Calibration Table. Individual Baseline values can be adjusted by directly editing the values in the Baseline column of the Calibration Table. When the Fixed Intercept? control is checked, the linear fit is forced to intersect with the y-Intercept at an OD Scanner reading of zero. It is recommended that you keep the Fixed Intercept? control checked, unless you have entered Reference values that span both the low and high range of the biomass units into which you are calibrating. When the Intercept is fixed, generally it is recommended to keep it fixed at zero, unless the reference method has a built-in offset that you haven't already accounted for (e.g. if you measured OD in a complex medium and didn't zero the spectrophotometer using the media alone, then you should enter the OD of the medium alone as the Intercept value).

STEP 4: Enter the type of reference data into the "Calibration Units" box at the top left of the graph. It is recommended that you include means of identifying both the organism and the reference method (e.g. "e coli g/L"). But be aware that if you type in a name that is longer than 10 characters (including spaces), it will automatically be truncated to 10 characters.

STEP 5: Choose the data transform method. Generally the "Linear-Linear" is recommended. If your samples were collected

at logarithmic intervals, the "Log-Log" transform may work best. However, be aware that an error will be reported if the baseline-corrected Bug Units value is less than or equal to zero.

STEP 6: Choose the Polynomial order that will be used to fit the data. We recommend using the lowest polynomial order that adequately fits the data. Due to the high linearity of the BE2100 sensor response to biomass, a linear fit (polynomial order = 1) is generally recommended. The root mean-squared (RMS) error and linear correlation coefficient (R^2) for the fit are shown below the graph. **RMS Error** indicates the root mean squared difference between the linear fit and the actual data, so the smaller the RMSE, the better the fit. **R^2** values can range between 0 and 1, with 1 indicating perfect linear correlation. The polynomial coefficients ("Poly Coeff") resulting from the fit are also displayed below the graph. The coefficients are listed from lowest to highest polynomial order: offset, linear, quadratic, and cubic.

STEP 6: If you are satisfied with the fit, select the "Accept Fit" button. You will be prompted to choose a path and name for the calibration file to be saved. Following file saving, the new calibration is automatically written into sensor memory. The calibration coefficients will be remembered across power cycles. The instrument can now be run in calibrated mode, without the need to be hooked up to a computer.

Running in Calibrated Mode

Now that you have saved a calibration into sensor memory, if you wish to collect calibrated data, you simply need to turn On calibration. Calibration can be turned On within the Virtual Instrument software from the "User Cal." column of the Device Configuration table (tab at the top of the screen). Alternatively, the keypad interface on the Base Unit can be used to turn User Calibration ("Cal") On or Off.

Note: When running in calibrated mode, the "Error Code Interpretation" column of the "Biomass Readings" table will display a warning message if the raw sensor data falls outside the calibrated range. In the event of this condition, the message "Warning: Extrapolating Beyond Calibration" will be displayed.

DATA VIEWER

Opening, Viewing, and Resaving Data Files

A separate "BE2x00 Data Viewer" program is provided for displaying previously acquired data. The overall organization and appearance of the program is much like that of the "BE2x00 Virtual Instrument". Previously acquired files are opened (using the "Open" button) from within the "File" tabs, located in the bottom half of the program window. Files can be re-saved (using the "Resave" button), but a new filename must first be selected. A suggested filename is automatically generated, that consists of the original filename appended with the date and time at which it was resaved. Resaving with the original filename is disabled in order to protect against accidental overwriting of data. This is particularly important when the Data Viewer program is used to open a file into which data is still actively being acquired (by the "Virtual Instrument" program). The data that is displayed in the Data Viewer program is a copy of the data that was present at the time the file was opened (the viewed data file is not automatically updated as new data points are collected). As with the Virtual Instrument program, the last tab in the files tabs contains a graph in which data from multiple files can be overlaid.

The configuration tabs in the top half of the Data Viewer screen are also similar to those within the Virtual Instrument program. However, in the Data Viewer, the configuration settings cannot be modified ("read-only"). These configuration settings are as read from the header of the data files that have been opened.

Retrospective Baseline Adjustment

The Data Viewer program also provides the capability to retrospectively adjust the baseline that is applied to the data. This feature is useful when you want to apply the same baseline setting across the entire file. If the baseline was changed one or more times during data acquisition (with the Virtual Instrument program), the changes were applied prospectively. In such cases, different segments of data have different baseline settings. The retrospective baseline adjustment feature allows you to equalize the baseline setting across all data segments.

> **STEP 1:** From within one of the "File" tabs, press the "Open" button, select the file you want to work on, and then press "OK".

STEP 2: From within the same "File" tab, press the "Adjust Base." button. The "Set Baseline Retrospectively.vi" window will open.

STEP 3: Set the "Baseline Method" control to one of the three settings:

"Set manually": If you select this option, you should type the new baseline value directly into the "Baseline Value" control.

"Retrieve from config. table": If you select this option, the value that is in the "Device Configuration" table (in the top half of the program window) is written into the "Baseline Value" control.

"Compute from graph": If you select this option, the baseline value is determined by averaging all points between the "Baseline Start" and "Baseline End" annotations. If you have not already created these annotations, you can do so by hitting the "Cancel" button, right-clicking over the graph where you want to start the baseline averaging, selecting "Create Annotation" >> "Baseline Start/End".

STEP 4: If the "Baseline Correction" control if set to Off, turn it On. Press the "OK" button to put your changes into effect. If you want to save your changes to file, press the "Resave" button, select a new filename, and then press the "OK" button.

Note 1: Whenever changes are made, the "Unsaved Changes" button in the "Software Settings" tab is activated (turns from grey to red). If you try to exit the program without saving your changes, you will first be prompted to make sure you are aware that your changes have not been saved.

Note 2: The Data Viewer program does not communicate with BE2x00 instruments. Changing the baseline setting from within the Data Viewer program affects only the data read from and/or saved to file, but does not affect the baseline settings stored in sensor memory. If you want to change the instrument settings, the "BE2x00 Virtual Instrument" program should be used instead.

Retrospective Calibration Adjustment

The Data Viewer program also provides the capability to retrospectively adjust the user calibration that is applied to the data. This feature may be useful if the calibration settings were changed midway through a fermentation run. During data acquisition (using the Virtual Instrument program) such changes are applied prospectively. The Data Viewer program allows you to apply the calibration uniformly across the entire data file. In other situations you may wish to see the effect of applying different calibrations to the same data set.

STEP 1: From within one of the "File" tabs, press the "Open" button, select the file you want to work on, and then press "OK".

STEP 2: From within the same "File" tab, press the "Adjust Cal." button. The "Set Calibration Retrospectively.vi" window will open.

STEP 3: Set the "Calibration Update Method" control to one of the four settings:

"Set Manually": If you select this option, you should type the new calibration settings directly into the "Calibration Settings" control. This option is useful in circumstances in which you have already performed a calibration fit in an external program (e.g. Microsoft Excel).

"Retrieve from Cal. Table": If you select this option, the settings that are currently in the "User Calibration" table (in the top half of the program window) are written into the "Calibration Settings" control.

"Retrieve from Cal. File": This option is useful if you have previously saved a calibration file, and want to apply the same calibration settings to the currently open data file.

"Open Cal. Window": Selecting this option will open the *User Calibration* window. This is the same window as is provided in the BE2x00 Virtual Instrument program. See the section "Editing, Generating, and Saving a Calibration" for a full description of how to operate the controls in this window.

Note: To view calibrations without having to first open a data set, use the "Cal Window" button provided in the "User Calibration" tab in the top portion of the program window. Within this

window you can modify and save new calibrations without applying these changes to a data file.

STEP 4: If the "Calibration" control if set to Off, turn it On. Press the "OK" button to put your changes into effect. If you want to save your changes to file, press the "Resave" button, select a new filename, and then press the "OK" button.

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*BE*2100 Sensor Specifications

Sensor (Performance)

Range of OD Sensitivity	0 to >300 OD units
OD Accuracy (typical RMSE in user-calibrated mode)	15% (OD 0.5-300) 0.1 OD (OD <0.5)
Averaging Time Constant	3 sec - 8 min
Performance Verification/Recalibration	calibration cups (2)
Calibration to external reference standards	via user interface software

Sensor (Electrical)

DC Power In	5V, 90 mA
Certifications	CE marked. Tested for compliance to EMC standards EN55011 and EN61000, and safety standard EN 61010.
Sensor communication with base unit or *BE|USB*	Serial digital (protocol available on request)
Sensor Cable	
Connector	BE2100 sensor, male (4-wire, threaded)
Length	
Standard	2m (6')
Extension cable (avail. as accessory)	4m (12')

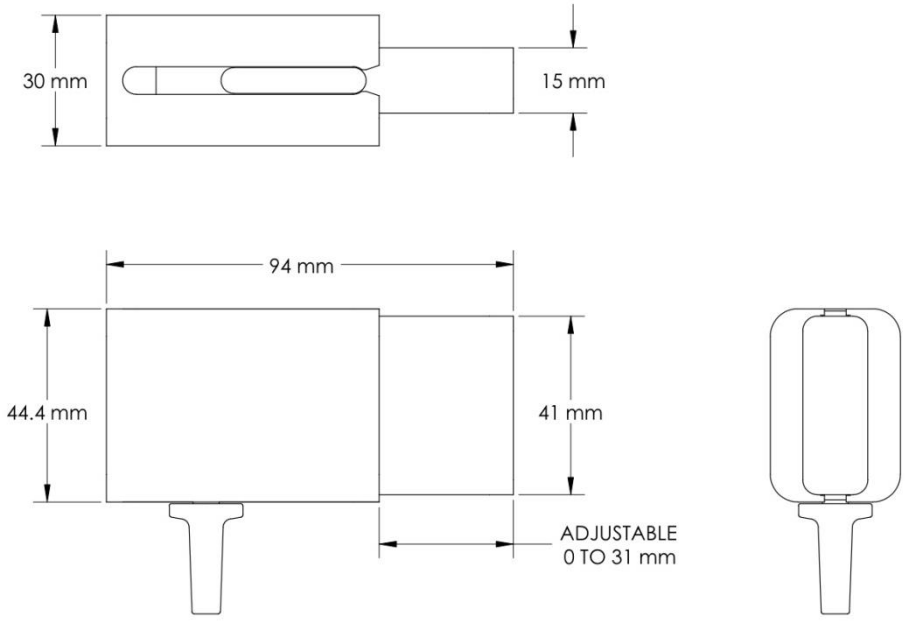
Sensor (Environmental and Safety)

Operating Temperature	0 to 50ºC (30 to 120F)
Environmental Seals	Yes – Splash Proof
Laser Product Classification	1M

Sensor (Physical)

Front Face Width	15mm (0.59”)
Front Face Height	41mm (1.61”)
Overall Length (excluding latch)	100mm (3.9”)
Active Optical Window (length x width)	25mm (1.0”) x 6mm (0.23")
Min. Vessel Diameter	5.3cm (2.1”)
Max. Vessel Diameter (std. Strap)	34cm (13.5”)
Max. Vessel Diameter (extended Strap)	Unlimited
Max. Window Depth	31mm (1.2”)
Functions with Flat Surfaces	Yes
External Materials:	
Body	Aluminum
Gasket	PVC
Filter	Acrylic
Strap	Nylon
Latch	Stainless Steel
Fasteners	Stainless Steel
Cable, Strain Relief	Polyurethane

*BE*2100 Sensor Dimensions



*BE*2100 Base Unit Features

Base Unit (Features)

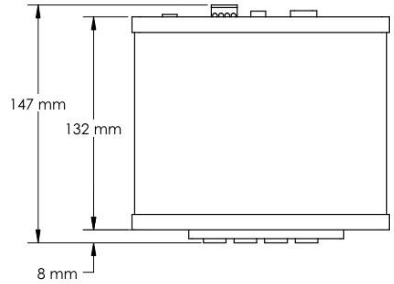
Access to individual sensor and base unit settings through interactive Keypad.
Password control (optional) of keypad access.
Lighted display with variable brightness control.
Digital (USB and RS-232) and analog (4-20mA or 2-10V) outputs (biomass and growth rate).

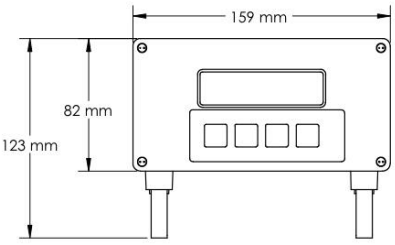
User Interface Software (Features)

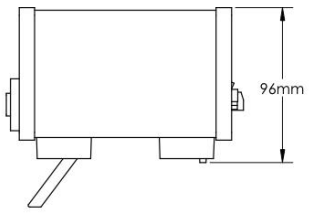
Real-time graphical and numerical display for the sensor.
Event marking on graph, both pre-defined and user-defined.
Baseline setting and subtraction.
User calibration of sensor output to any reference units.
Access to all sensor and base unit settings.

User Interface Software (Requirements)

Windows XP / Vista / 7 / 8 (32 or 64 bit) Operating System.
Minimum of 256 MB of RAM.
Minimum 200 MB free hard disk space.
1024 by 768 resolution (or higher) video adapter.
Available USB or 9-pin RS-232 COM port.
Microsoft-compatible mouse.
CD reader (required only at time of software installation).







*BE*2100 Base Unit Specifications

Base Unit (Electrical)

DC Power In (Both US and International plug adapters available)	9V, 1A
Certifications	CE marked. Tested for compliance to EMC standards EN55011 and EN61000, and safety standard EN 61010.
Sensor Input:	One *BE*2100 sensor
Connector	*BE*2100 sensor, female (4-wire, threaded)
Analog Output:	4-20mA (500Ω max.) or 2-10V (using provided 500 Ω resistors)
Resolution	12 bits (0.004 mA or 2 mV)
Range Settings	6 settings, logarithmically spaced: 0.01-1000
Number of Outputs	4 ('+' and '–' screw terminal positions for sensor signal and exponential growth rate)
Digital Output	RS-232/DB9 and USB
Communications Cable (optional):	
Connectors	DB9 (M/F) or USB (A/B)
Length	
Standard	2m (6')
Custom	up to 15m (50')

Base Unit (Physical)

Overall Width	15.9cm (6.2")
Overall Length (without connectors)	13.2cm (5.2")
Overall Height (without feet)	8.2cm (3.2")

Base Unit (Environmental)

Operating Temperature	0 to 40ºC (30 to 100F)
Operating Environment	dry location use only

*BE*2400 Base Unit Features

Base Unit (Features)

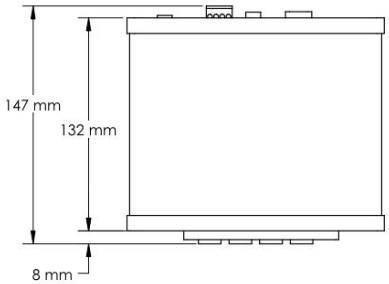
Access to individual sensor and base unit settings through interactive Keypad.
Password control (optional) of keypad access.
Lighted display with variable brightness control.
Digital (USB and RS-232) and four analog (4-20mA or 2-10V) outputs.

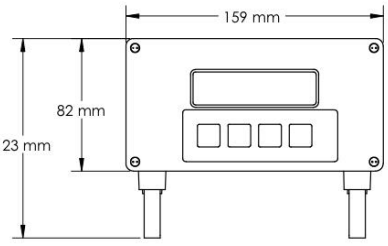
User Interface Software (Features)

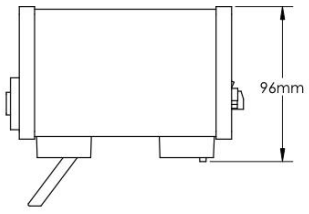
Real-time graphical and numerical display for each sensor.
Event marking on graph, both pre-defined and user-defined.
Baseline setting and subtraction.
User calibration of sensor output to any reference units.
Access to all sensor and base unit settings.

User Interface Software (Requirements)

Windows XP / Vista / 7 / 8 (32 or 64 bit) Operating System.
Minimum of 256 MB of RAM.
Minimum 200 MB free hard disk space.
1024 by 768 resolution (or higher) video adapter.
Available USB or 9-pin RS-232 COM port.
Microsoft-compatible mouse.
CD reader (required only at time of software installation).







*BE*2400 Base Unit Specifications

Base Unit (Electrical)

DC Power In (Both US and International plug adapters available)	6V, 1A
Certifications	CE marked. Tested for compliance to EMC standards EN55011 and EN61000, and safety standard EN 61010.
Sensor Input:	Up to 4 *BE*2100 sensors
Connector	BE2100 sensor, female (4-wire, threaded)
Analog Output:	4-20mA (500Ω max.) or 2-10V (using provided 500 Ω resistors)
Resolution	12 bits (0.004 mA or 2 mV)
Range Settings	6 settings, logarithmically spaced: 0.01-1000
Number of Outputs	8 ('+' and '–' screw terminal positions for each sensor)
Digital Output	RS-232/DB9 and USB
Communications Cable (optional):	
Connectors	DB9 (M/F) or USB (A/B)
Length	
Standard	2m (6')
Custom	up to 15m (50')

Base Unit (Physical)

Overall Width	15.9cm (6.2")
Overall Length (without connectors)	13.2cm (5.2")
Overall Height (without feet)	8.2cm (3.2")

Base Unit (Environmental)

Operating Temperature	0 to 40ºC (30 to 100F)
Operating Environment	dry location use only

BE│USB Features and Specifications

User Interface Software Features (CD included with *BE*│USB)

Real-time graphical and numerical display of sensor data.
Event marking on graph, both pre-defined and user-defined.
Baseline setting and subtraction.
User calibration of sensor output to any reference units.
Access to all sensor settings.

User Interface Software Requirements

Windows XP / Vista / 7 /8 (32 or 64 bit) Operating System.
Minimum of 256 MB of RAM.
Minimum 200 MB free hard disk space.
1024 by 768 resolution (or higher) video adapter.
Available USB or 9-pin RS-232 COM port.
Microsoft-compatible mouse.
CD reader (required only at time of software installation).

***BE*│USB (Electrical)**

DC Power In	5V, 90mA
Digital Output	USB
Connectors	USB-B female and *BE*2100 sensor female (4-wire threaded)
USB Communications Cable (included)	
Connectors	USB-A male (computer) and USB-B male (*BE*|USB)
Length	
Standard	2m (6')
Custom	up to 15m (50')

***BE*│USB (Physical)**

Overall Width	2.8cm (1.1")
Overall Length	5cm (2")
Overall Height	2.3cm (0.9")

***BE*│USB (Environmental)**

Operating Temperature	0 to 40ºC (30 to 100F)
Operating Environment	dry location use only

Appendix II. Description of Base Unit Display Menu and Functions

i. Startup screen 1 (displayed at startup for 5 seconds):

					B	**u**	**g**	**L**	**a**	**b**		**L**	**L**	**C**				
	M	**o**	**n**	**.**		**S**	**W**		**V**	**e**	**r**	**.**		**x**	**x**	**x**	**x**	**x**	**x**

ii. Startup screen 2 (displayed for 5 seconds following startup screen):

							B	**u**	**g**	**E**	**y**	**e**						
S	**e**	**n**	**s**	**.**		**S**	**W**		**V**	**e**	**r**	**.**		**x**	**x**	**x**	**x**	**x**	**x**

For BE2400 (mux) base units, the startup screen will be repeated for each of the (up to 4) ports to which a sensor is attached. If no sensor is connected, screen 2 will display the following and the monitor will check periodically for sensor connection. If a sensor is connected subsequently, the sequence will start from step 1.a.ii above:

S	**e**	**n**	**s**	**o**	**r**		**D**	**i**	**s**	**c**	**o**	**n**	**n**	**e**	**c**	**t**	**e**	**d**	

If the above message is displayed when working with a BE2400 base unit but a sensor is connected, the instrument may be attempting to connect to a sensor port that is disconnected. Press any of the buttons on the keypad and select "Scrol" to make the display scroll through all available sensors. Or select "Lock" and then choose an available sensor to which you want to lock the display.

iii. After the startup timer expires the screen reverts to the Normal Operating screen:

1. Line 1: Reserved for error/warning messages (see below)
   If keypad access is blocked due to remote operation by a PC, line 1 will display "Remote Operation". If errors or warnings are detected, then the error/warning message will be displayed (2 seconds duration) alternating with the "Remote Operation" message (3 seconds duration).
2. Line 2: Sensor readings
   a. If the 'Cal' setting is off:

		x	**x**	**x**	**.**	**x**	**x**		**B**	**u**	**g**		**U**	**n**	**i**	**t**	**s**	

   b. If the 'Cal' setting is on:
      The display format and units will be determined by the user (e.g. "g/L E. coli").

   The monitor will poll the sensor approximately once per second and update the screen with the latest information received. The letter 'C' will appear at the end of the second line of the display when baseline correction has been turned On.

   On BE2400 base units, there is the option of scrolling the display between all attached sensors, or locking to the display to one particular sensor. When in scrolling mode, the results for each sensor are sequentially displayed for approximately 5 seconds each.

iv. Error and warning messages:
    1. Warnings
        a. If ambient light is higher than desirable but is not preventing a valid measurement, then line 1 will read “High Ambient Light”, and line 2 will continue to report the quantitative result.
        b. If the calibration switch is set to the “On” position, and a user calibration has been stored, but extrapolation is required in order to report the result, line 1 will read “Extrapolating Cal”, and line 2 will continue to report the quantitative result.
    2. Error conditions
        a. If ambient light is high enough to prevent a valid measurement, then line 1 will read “Signal Saturated”, and line 2 will be blank.
        b. If the sensor is disconnected from the base unit, then line 1 will read “Sensor Disconnected” and line 2 will be blank.
        c. If the sensor measures signals that are below the internal calibration range, then line 1 will read “Below Range”.
        d. If the sensor measures signals that are above the internal calibration range, then line 1 will read “Above Range”.
        e. If there is an error converting the data into BugUnits, then line 1 will read “Data Error”, and line 2 will be blank.

Additional errors and warnings are shown in the table on the following page.

Error and Warning Codes

Code	Type of Event	Useage[1]	Condition	Notes
-10	Error	C	Check Sum Error	Sent check sum does not match computed check sum.
-9	Error	C	Value out of range	This is a serial communication error where the data packet following a command contains values outside of the allowed range.
-8	Error	C	Network busy	This general error code applies when any of the 3 communicators (sensor, monitor, or PC) is busy with another task when any other of the communicators is attempting to talk with it.
-7	Error	G	User Calibration Error	Attempted to fit negative or zero-valued data in log-log space.
-6	Error	G	User Calibration Error	Insufficient number of calibration points to compute calibration coefficients.
-5	Error	D, G	Signal Saturated	Ambient light is so high that it is preventing measurement.
-4	Error	D, G	Sensor Disconnected	Sensor not plugged into monitor.
-3	Error	D, G	Below Range	Signal is below the internal cal. range
-2	Error	D, G	Above Range	Signal is above the internal cal. range
-1	Error	D, G	Data Error	Error converting the individual detector data into BugUnits.
0	None	D, G	Normal Operation	
1	Warning	D, G	High Ambient Light	Ambient light is high, but a measurement can still be made
2	Warning	D, G	No Calibration	The calibration switch is “on”, but no calibration data is available.
3	Warning	D, G	Extrapolating Cal	The measurement is outside of the user-calibration range.

Notes for Table 1:
(1) D = base unit display, G = graphical user interface, C = serial communication error.

v. 4-key communication:
    1. The variables and functions, in the order in which they will appear on the menu are:
        a. Scroll/Lock (BE2400 base unit only): In scroll mode the results for attached sensors are sequentially displayed for 5 seconds each. In locked mode, only the results for the selected sensor are displayed.
        b. Baseline ("Base"): The user will have the ability to set a baseline and turn baseline correction "On" or "Off". In the "On" state, the most recently set baseline is subtracted from the Bug Units. In the "Off" state, the baseline is ignored. The default baseline setting is 'Off'.
        c. Calibration Switch ("Cal"): The user will have the ability to view results in either "calibrated" or "un-calibrated" mode. The default 'Calibration Switch' setting is 'Off'.
        d. Sensor Check ("Chk"): The user will be able to verify that the sensor is working correctly by placing standard "calibration cups" onto the sensor, and, if necessary, change the sensor calibration coefficients (offsets and gains).
        e. Averaging time constant ("Ave"): Determines the time response of the sensor to changing signals. The default averaging time constant is 2 minutes.
        f. Range for Analog Output 1 ("AO1" on BE2100; "Rnge" on BE2400): Determines the signal level corresponding to the maximum current (20 mA) on analog output 1. The default analog output 1 range is 100.
        g. Range and Time Window for Analog Output 2 ("AO2"; BE2100 base units only): The "Range" setting determines the rate of change (per hour) corresponding to the maximum current (20 mA) on analog output 2. The default analog output 2 range is 100. The "Wndw" setting determines the time window over which the growth rate is computed for analog output 2. The default setting is 8 min.
        h. Comm port switch ("Comm"; BE2100 base units only): Determines whether the RS-232 or USB port is active on the monitor. The default setting is 'USB'.
        i. Vacuum Fluorescent Display (VFD) brightness level ("Disp"): The VFD brightness can be adjusted between 4 levels. The default VFD brightness level is 3.
        j. Password Protection ("Pswd"): Allows the user to set a password and lock others out of changing settings unless they enter the correct password.
        k. Reset: Resets all of the above adjustable parameters to their factory-default settings
    2. Settings b-e are stored in sensor memory; and settings a and f-j are stored in base unit memory. The settings are maintained across power-cycling of the instrument.

vi. If any of the Soft-keys are pressed during Normal Operation, the system configuration menu is activated.
    1. Scroll/Lock (BE2400 Base Units only): The opening screen of the BE2400 configuration menu provides the option of putting the display into scrolling or locked display mode, or proceeding to the sensor-specific configuration menus.
        a. "Scrol": The display will sequentially scroll between all available sensors at 5 second intervals.

b. "Lock": The display will be locked to one particular sensor. An additional screen is displayed to allow selection of the sensor to which the display will be locked.
c. "More": Provides entry into the sensor-specific configuration menus. An additional screen is displayed to allow selection of the sensor to which the display will be locked.

2. Sensor-specific settings
   a. Baseline Mode ("Base"): If the button below "Base" is pressed, the Baseline Correction mode is entered:

	B	**a**	**s**	**e**	**l**	**i**	**n**	**e**		**O**	**p**	**t**	**i**	**o**	**n**	**s**	**:**			
	O	**n**			**S**	**t**	**a**	**r**	**t**		**S**	**h**	**o**	**w**		**B**	**a**	**c**	**k**	
	△				△						△					△				

      i. 'On': The button under 'On' toggles baseline correction between 'On' and 'Off'. In the 'On' state, the baseline is used to correct the value displayed during normal operation. Once pressed, the Baseline 'On' or 'Off' command is sent to the sensor (no further user confirmation is required).
      ii. 'Start': Pressing the button under ''Start' begins the baseline data collection process. Once pressed, the word 'Start' will be replaced with 'End'. Baseline data collection continues until either the 'End' or the 'Back' button is pressed. If the 'End' button is pressed, the new baseline value is displayed on the VFD for 3 seconds, after which the above Baseline Mode screen is then again displayed. The new baseline value is saved in the sensor (no further user confirmation is required).

	B	**a**	**s**	**e**	**l**	**i**	**n**	**e**		**S**	**e**	**t**	**t**	**i**	**n**	**g**	**:**			
		x	**x**	**x**	**.**	**x**	**x**		**B**	**u**	**g**		**U**	**n**	**i**	**t**	**s**			

      iii. 'Show': Pressing the 'Show' button results in the current baseline value being displayed on the VFD for 3 seconds (same screen as if the End button was pressed in step b, above). If 'Show' is pressed while gathering is in progress the top line of the display will read "Sensor Busy"
      iv. 'Back': The 'Back' button allows the user to step back in the menu.
   b. Calibration Mode ("Cal"): If the button below "Cal" is pressed, the Calibration mode is entered:

C	**a**	**l**	**i**	**b**	**r**	**a**	**t**	**i**	**o**	**n**		**O**	**p**	**t**	**i**	**o**	**n**	**s**	**:**	
	O	**n**			**H**	**e**	**l**	**p**								**B**	**a**	**c**	**k**	
	△				△						△					△					

      The buttons in this mode will have the same functions as in the baseline mode.

c. Sensor Check ("Chk"): If the button below the 'Chk' is pressed, the Sensor Check mode will be entered.

S	**e**	**n**	**s**	**o**	**r**		**C**	**h**	**e**	**c**	**k**		**M**	**o**	**d**	**e**	**:**	
	R	**u**	**n**			**H**	**e**	**l**	**p**							**B**	**a**	**c**	**k**

i. 'Back': If the 'Back' button is pressed (here and in all of the following screens in Sensor Check mode, unless otherwise noted) the previous main configuration menu will be displayed again.

ii. 'Run': Pressing the button under 'Run' begins the following programming sequence:

	A	**t**	**t**	**a**	**c**	**h**		**L**	**o**	**w**		**C**	**a**	**l**		**C**	**u**	**p**	**.**
R	**e**	**a**	**d**	**y**		**H**	**e**	**l**	**p**							**B**	**a**	**c**	**k**

iii. 'Ready': Pressing the 'Ready' button leads to 25 measurements (~1 sec. each) being made. The mean and standard deviation are computed.

During the measurement the base unit displays the message:

M	**e**	**a**	**s**	**u**	**r**	**i**	**n**	**g**	**.**	**.**	**.**		**X**		**o**	**f**		**2**	**5**

where X is the current measurement number (1-24) and is updated following each measurement (except for the 25th).

iv. Once the 25 measurements are complete, the standard deviation (SD) is computed for each of the detector signals.

1. If the SD is less than Threshold 1 for all of the signals, the base unit displays:

C	**o**	**n**	**t**	**i**	**n**	**u**	**e**		**t**	**o**		**H**	**i**		**C**	**a**	**l**	**?**	
						Y	**e**	**s**				**N**	**o**						

a. If the "Yes" button is selected, the routine continues to the next screen.
b. If the "No" button is selected, the Sensor Check routine is terminated, and the previous Configuration screen is displayed.

2. Else, the monitor displays the message:

	U	**n**	**s**	**t**	**a**	**b**	**l**	**e**	**.**		**R**	**e**	**p**	**e**	**a**	**t**	**?**	
						Y	**e**	**s**				**N**	**o**						

a. If "Yes" is selected, the routine continues back at step c.ii.
b. If "No" is selected, the routine is terminated, and the previous Configuration screen is displayed.

v. High Cal Cup: If the "Low Cal Cup" measurement is successfully completed, the "High Cal Cup" screen is next displayed:

A	**t**	**t**	**a**	**c**	**h**		**H**	**i**	**g**	**h**		**C**	**a**	**l**		**C**	**u**	**p**	**.**
R	**e**	**a**	**d**	**y**		**H**	**e**	**l**	**p**							**B**	**a**	**c**	**k**

The subsequent "High Cal Cup" screen sequences will be the same as those shown above for the "Low Cal Cup" sequence.

vi. If both the "Low Cal" and "High Cal" measurements are successfully completed, they will be converted into "Bug Units". Then the absolute value of the errors for the cal cup measurements are computed relative to the expected value.

vii. If the absolute errors are less than or equal to Thresholds 3 and 4, respectively, for the "Low Cal" and "High Cal" measurements, the monitor displays:

			S	**e**	**n**	**s**	**o**	**r**		**P**	**a**	**s**	**s**	**e**	**d**	**.**			
U	**p**	**d**	**a**	**t**	**e**						**H**	**e**	**l**	**p**		**B**	**a**	**c**	**k**

1. If the user selects 'Update', the new offsets and gains are determined based on the low and high cal cup measurements. The low cal cup reading on each detector is the offset value for each detector. The high cal cup reading on each detector is divided into a reference reading for each detector (stored in sensor memory), to determine the gain for each detector. The new sensor calibration coefficients are then written to memory.
2. The 'Back' button allows the user to step back in the menu.

viii. Otherwise, the monitor displays the message:

			S	**e**	**n**	**s**	**o**	**r**		**F**	**a**	**i**	**l**	**e**	**d**	**.**			
U	**p**	**d**	**a**	**t**	**e**						**H**	**e**	**l**	**p**		**B**	**a**	**c**	**k**

ix. The 'Update' and 'Back' functions produce the same result as in the 'Sensor Passed' case (section h, above).

d. Averaging Time Constant Selection ("Ave"): Pressing "Ave" button displays the Time Constant selection menu:

T	**i**	**m**	**e**		**C**	**o**	**n**	**s**	**t**	**a**	**n**	**t**		**S**	**e**	**l**	**e**	**c**	**t**
	S	**e**	**t**			**H**	**e**	**l**	**p**		**S**	**h**	**o**	**w**		**B**	**a**	**c**	**k**

i. "Set": Pressing the "Set" button brings up the following screen:

S	**e**	**l**	**e**	**c**	**t**		**T**	**i**	**m**	**e**		**C**	**o**	**n**	**s**	**t**	**a**	**n**	**t**
0	**S**	**e**	**c**		**3**	**0**	**S**	**e**	**c**		**M**	**o**	**r**	**e**		**B**	**a**	**c**	**k**

ii. Pressing the "More" button brings up screens with additional time constants. Pressing the button beneath any of the times

will set that as the new time constant. "2min" is the default setting.

iii. Selecting the 'Show' key brings up a screen in which the current setting is displayed.

e. AO1 Range: Pressing the button below "AO1" (for BE2100 base units) or "Range" (for BE2400 base units) brings up the Analog Output1 Range menu:

A	**n**	**a**	**l**	**o**	**g**		**O**	**u**	**t**	**p**	**u**	**t**	**1**		**R**	**a**	**n**	**g**	**e**
	S	**e**	**t**			**H**	**e**	**l**	**p**		**S**	**h**	**o**	**w**		**B**	**a**	**c**	**k**

i. "Set": Pressing the "Set" button allows the user to toggle between the allowable selections for this field ('0.01', '0.1', '1.0', '10', '100', '1000'; where '100' is the default setting).

ii. "Show": Selecting the "Show" key brings up a screen displaying the current range value for 3 sec, after which the screen returns to the main AO1 Range screen.

f. AO Range 2 ("AO2"; BE2100 base units only): If the button below 'AO2' is pressed , the Analog Output2 Menu is displayed:

A	**n**	**a**	**l**	**o**	**g**		**O**	**u**	**t**	**p**	**u**	**t**	**2**		**M**	**e**	**n**	**u**	**:**
R	**a**	**n**	**g**	**e**		**W**	**n**	**d**	**w**							**B**	**a**	**c**	**k**

i. "Range": Pressing "Range" button brings up the following screen:

A	**n**	**a**	**l**	**o**	**g**		**O**	**u**	**t**	**p**	**u**	**t**	**2**		**R**	**a**	**n**	**g**	**e**
	S	**e**	**t**			**H**	**e**	**l**	**p**		**S**	**h**	**o**	**w**		**B**	**a**	**c**	**k**

This menu is analogous to that for the AO1 range described above.

ii. "Wndw": Pressing the 'Wndw' button brings up the following screen:

S	**l**	**o**	**p**	**e**		**T**	**i**	**m**	**e**		**W**	**i**	**n**	**d**	**o**	**w**	**:**		
	S	**e**	**t**			**H**	**e**	**l**	**p**		**S**	**h**	**o**	**w**		**B**	**a**	**c**	**k**

1. "Set": Pressing the "Set" button allows you to select the growth rate time window (allowed values are '1min', '2min', '4min', '8min', '16min', '32min'; '8min' is the default setting.). This setting determines the range of time over which fitting is performed when estimating the exponential growth rate constant.
2. "Show": Selecting the "Show" key brings up a screen in which the current setting is displayed for 3 sec.

g. Communication Select ("Comm", BE2100 base units only): Pressing the button below "Comm" brings up the following screen:

C	**o**	**m**	**m**	**u**	**n**	**i**	**c**	**a**	**t**	**i**	**o**	**n**		**s**	**e**	**l**	**e**	**c**	**t**
	U	**S**	**B**		**R**	**S**	**2**	**3**	**2**		**S**	**h**	**o**	**w**		**B**	**a**	**c**	**k**

i. Selecting “USB” or “RS232” results in that communication port becoming active. The default setting is “USB”. When “USB” is selected, RS-232 communication will still function as long as the USB cable is not also plugged in. When “RS232” is selected, USB communication is disabled.

ii. “Show”: Selecting the ‘Show’ key brings up the following screen for 3 sec, after which the screen returns to the main ‘Communication select’ screen:

C	**u**	**r**	**r**	**e**	**n**	**t**		**a**	**c**	**t**	**i**	**v**	**e**		**c**	**o**	**m**	**m**	**=**
X	**X**	**X**	**X**	**X**		**(**	**d**	**e**	**f**	**a**	**u**	**l**	**t**	**=**	**U**	**S**	**B**	**)**	

where XXXXX is either ‘USB’ or ‘RS232’.

h. “Disp”: If the button below “Disp” is pressed, the Display Brightness menu is shown:

D	**i**	**s**	**p**	**l**	**a**	**y**		**b**	**r**	**i**	**g**	**h**	**t**	**n**	**e**	**s**	**s**	**:**	
	S	**e**	**t**		**H**	**e**	**l**	**p**			**S**	**h**	**o**	**w**		**B**	**a**	**c**	**k**

i. “Set”: Pressing the “Set” button allows you to toggle between the allowable selections for the display brightnes (‘1’, ‘2’, ’3’, and ’4’; ‘3’ is the default setting) as follows:

B	**r**	**i**	**g**	**h**	**t**	**n**	**e**	**s**	**s**		**l**	**e**	**v**	**e**	**l**		**=**		**X**
		-					**+**									**B**	**a**	**c**	**k**

Selecting the ‘+’ or ‘-‘ keys respectively raises or lowers the brightness level and changes the displayed value of brightness (X = 1-4). If the display is already at its maximum setting, pressing the ‘+’ key has no effect; likewise for the ‘-‘ key when the display is already at the minimum brightness setting. Selecting the ‘Back’ button brings up the main “Display Brightness” screen.

ii. “Show”: Selecting the “Show” key brings up the following screen for 3 sec, after which the screen returns to the main ‘Display brightness’ screen:

C	**u**	**r**	**r**	**e**	**n**	**t**		**b**	**r**	**i**	**g**	**h**	**t**	**n**	**e**	**s**	**s**		**=**
	X			**(**	**d**	**e**	**f**	**a**	**u**	**l**	**t**		**=**		**3**	**)**			

i. “Pswd”: If the button below “Pswd” is pressed, the Password Protection menu is displayed:

	P	**a**	**s**	**s**	**w**	**o**	**r**	**d**		**P**	**r**	**o**	**t**	**e**	**c**	**t**	**:**		
	O	**n**				**S**	**e**	**t**								**B**	**a**	**c**	**k**

i. “On”: Pressing the “On” button toggles between the On and Off states of password protection. The default setting is Off. When password protection is On, keypad entry into the configuration menus is password-protected, as shown below:

		E	**n**	**t**	**e**	**r**		**p**	**a**	**s**	**s**	**w**	**o**	**r**	**d**	**:**			
		1					**2**					**3**					**4**		

△ △ △ △

If the password is not successfully entered within 10 seconds the display reverts to 'Normal' operation. If you attempt to turn on password protection without first setting a password, the following screen is displayed for 3 seconds, followed by a return to the "Password Protect:" menu:

P	**a**	**s**	**s**	**w**	**o**	**r**	**d**		**m**	**u**	**s**	**t**		**b**	**e**		**S**	**e**	**t**
b	**e**	**f**	**o**	**r**	**e**		**s**	**e**	**l**	**e**	**c**	**t**	**i**	**n**	**g**		**O**	**n**	**.**

If you are locked out of keypad access, the BE2x00 Virtual Instrument software provides a command for turning Off the password protection (see ***Working with the BE2100 and BE2400 Base Units: Password Locking and Unlocking***).

ii. 'Set': Pressing the 'Set' button brings up the following screen:

E	**n**	**t**	**e**	**r**		**n**	**e**	**w**		**6**	**-**	**d**	**i**	**g**	**i**	**t**		**P**	**W**
		1					**2**					**3**					**4**		

If 6 digits are not entered within 15 seconds, the display will revert to the "Password Protect:" screen. Otherwise, you will be prompted to re-enter the new 6-digit password. If the password is successfully re-entered, the screen will display the message "New password accepted".

j. "Reset": Pressing the "Reset" button bring up the following menu:

R	**e**	**s**	**e**	**t**		**a**	**l**	**l**		**s**	**e**	**t**	**t**	**i**	**n**	**g**	**s**	**:**	
R	**e**	**s**	**e**	**t**		**H**	**e**	**l**	**p**							**B**	**a**	**c**	**k**

i. "Reset": Pressing the 'Reset' button brings up the following confirmation screen:

			C	**o**	**n**	**f**	**i**	**r**	**m**		**r**	**e**	**s**	**e**	**t**	**:**			
						Y	**e**	**s**				**N**	**o**						

Selecting "Yes" resets all variables to their factory default values (including the sensor gains and offsets). Selecting "No" returns the screen to the "Reset All Settings:" window without changing any settings.

Appendix III. Serial Protocol Specifications: Remote Connection to the BE2x00 Instrument via USB or RS-232

1. Scope

Applies to communication between the BE2100 Sensor and BE2100 Base Unit, BE2400 Base Unit, or BE|USB adapter; their communication with each other, as well as communication protocol with a host PC.

2. General Specifications

    1. Serial data is transmitted over either a RS-232 serial port or over USB.
    2. Serial data is transmitted at 19200 baud rate, No Parity, 8 data bits, 1 stop bit.
    3. Data transmission is non-streaming only.
    4. All data will be sent as a Command-Response pair. The response key is the lower case complement of the command key, except in the case where the command is not recognized.
    5. If a command is not recognized by the receiver then a special ASCII character ("!", hex: 0x21) is returned to the sender with a 1byte data packet.
    6. Empty command packets will be used to prompt for the current settings to be returned.
    7. Non-empty command packets are used to set the parameters for the specified field value, provided the packet and parameters for the field meet specifications. If the data in a non-empty command packet is successfully received, the response will include a duplication of the data in the command packet.
    8. If the data in a non-empty command packet is out of the allowed range of values, then the response will contain a 1 byte data packet with a value (-9) that indicates that the data was "Out of Range". If the computed check sum does not match the sent check sum, then the response will contain a 1 byte data packet with a value (-10) that indicates that there was "Check Sum Error". If data packet is both out range and contains a check sum error, the response data packet will indicate "Check Sum Error". In either case, other than responding with the error message, no action will be taken by the receiver in response to the command sent.
    9. Any 32-bit floating-point value is represented in IEEE 754 format unless otherwise specified.

3. Data Protocol (Packet)
    1. Serial data is sent in packets.
    2. The packet starts with a header byte, 0x5A (ASCII character 'Z').

3. The second byte in the packet is the length byte. Length byte is the total length of the data payload (min length of 0, max length of 127).
4. The third byte in the packet is the key byte (command and response keys provided in Tables 1-4).
5. The next byte(s) (up to but not including the checksum) are the data bytes. The data bytes can be zero bytes (empty packet) or up to 127 bytes of data. The length byte, described in 4.c above, refers to the total length of these data bytes.
6. The next-to-last byte is the checksum. The checksum chosen is a mod256 checksum byte, calculated using the key byte, data bytes and length byte. The checksum is formed by adding the hex-value of all bytes, and applying a modulus 256 to the sum.
7. The last byte is the footer byte, 0x3C (ASCII character '<').

4. Packet Send & Receive Specifications
    1. Each packet has a single message only.
    2. On Power-on of the Base Unit, the Base Unit will send a single Base Unit-version packet to the serial or USB port.
    3. If no sensor is connected at Base Unit power on, the Base Unit will send a single message of "No sensor connected" to the serial or USB port.
    4. On detection of a sensor, the Base Unit will send a single sensor-version packet to the serial or USB port.
    5. On detection of a sensor-disconnect status, the Base Unit will send a single message of "sensor disconnected" to the serial or USB port.

5. Data Protocol, Message Data
    1. Command keys recognized by all BE2x00 devices are shown on the left side of Table 1. The response keys to these commands are shown on the right side of Table 1. The commands are all ultimately received and responded to by a BE2100 sensor. When sent to a base unit or BE|USB adapter the commands are automatically relayed to and from the connected BE2100 sensor(s). Further details of these commands can be found in section 6, below.
    2. Command keys recognized BE2100 Base Units, BE2400 Base Units and and BE|USB adapters, but not by BE2100 Sensors, are shown on the left side of Table 2. The response keys are shown on the right side of Table 2.
    3. Comands recognized only by BE2100 and BE2400 Base Units are shown in Table 3.
    4. Commands recognized only by BE2400 Base Units are shown in Table 4.
    5. While the Base Unit menu configuration menu is active (activated by pressing any of the 4 keys on the pad), any commands sent to the Base Unit will be responded to with the lower case complement of the command plus a 1 byte data packet whose value indicates that the system is busy (error code = -8).
    6. Commands sent to the sensor while it is busy (e.g. in the middle of running the 'sensor check' routine or while it is in the middle of collecting

baseline data) will likewise be responded to with the lower case complement of the command sent plus a 1 byte data packet whose value indicates that the system is busy (error code = -8).

Table 1. Commands Recognized by the BE2100 Optical Sensor Head (and passed through by BE2100 Base Units, BE2400 Base Units, and BE|USB adapters when connected to sensors).

Key	PW	Command Description
A	R/W	Averaging Time Constant
B	R/W	Baseline Value
K	R/W	Start/Query/End Baseline
L	R/W	'Sensor Check' Function Control
M	R	Get data
O	R/W	On/Off settings for Base. Corr. and User Cal.
S	R	Sensor Serial Numbers
T	R/W	User Cal Units
U	R/W	User Cal Coefficients
V	R	Sensor embedded SW Version
W	R/W	Slope Window

Key	Return Data Description
a	Averaging Time Constant
b	Baseline Value
k	Baseline status
l	'Sensor Check' Function Status
m	Data output
o	On/Off settings for Base. Corr. and User Cal.
s	Sensor Serial Numbers
t	User Cal Units
u	User Cal Coefficients
v	Sensor embedded SW Version
w	Slope Window
!	Command not recognized

Table 2. Commands Recognized by BE2100 Base Units, BE2400 Base Units, and BE|USB adapters.

Key	PW	Command Description
@	R	Get Base Unit embedded SW version
#	R	Base Unit Serial Number

Key	Return Data Description
2	Base Unit embedded SW version
3	Base Unit Serial Number

Table 3. Commands Recognized only by BE2100 and BE2400 Base Units.

Key	PW	Command Description
J	R/W	Base Unit keypad lockout state
Q	R/W	Base Unit password set/reset/unlock
R	R/W	Range for Analog Out

Key	Return Data Description
j	Base Unit keypad lockout state
q	Base Unit password status
r	Range for Analog Out

Table 4. Commands Recognized only by BE2400 Base Units

Key	PW	Command Description
%	R/W	Sensor port switch state

Key	Return Data Description
5	Active sensor port

Notes for Tables 1-4:
(1) The symbols in the PW column indicate the type of access that is available: Read(R), Write(W), or both (R/W).

6. Command Descriptions

1. 'A' – 0x41 – Averaging Time Constant

The time constant (in seconds) used in the sensor for averaging the raw detector data. The value is represented as an unsigned integer16. Allowed values = 0, 30, 60, 120, 240, 480. Any non-allowed value entered is ignored. Default value is 120.

Key	Time Window	
1 Byte	2 Bytes	
'A' – 0x41		

Sending an empty-packet "A" command will return the "a" message, displaying the current setting:

Key	Time Window	
1 Byte	2 Bytes	
'a' – 0x61		

2. 'B' – 0x42 – Baseline Value

The offset value applied to the sensor ouput in order to compute the "Baseline-Corrected" sensor output. Values expressed as 32-bit floating-point number. Default is "0.0".

Key	Baseline to Set			
1 Byte	4 Bytes			
'B' – 0x42	LSB			MSB

Sending an empty-packet "B" command will return the "b" message, displaying the current setting:

Key	Current Baseline Value			
1 Byte	4 Bytes			
'b' – 0x62	LSB			MSB

3. 'J' – 0x4A – Base Unit Keypad Lockout

This command is used to lock (J value = 0x01) or unlock (J value = 0x00) access to the Base Unit keypad configuration menus. The default is unlocked. The state is always reset to unlocked at power up of the Base Unit. When a 'J1' (lock base unit) command is sent to a BE2400 (multiplexed) base unit, the base unit is automatically taken out of the scrolling state. The scrolling state prior to sending the 'J1' command is saved in memory, and automatically restored when the base unit is unlocked.

Key	J Value
1 Byte	1 Byte
'J' – 0x4A	

Sending an empty-packet "J" command will return the "j" message, displaying the currently set value:

Key	j Value
1 Byte	1 Byte
'j' – 0x6A	

When communicating with BE2100 or BE2400 base units, it is recommended that the base unit keypad be set to the "locked" state prior to performing any other communication steps. This will prevent conflict between serial communication commands and manually-initiated (keypad) access.

This command is only useful for BE2100 and BE2400 base units; in the case of the BE|USB adapter no keypad access is provided, so this command serves no purpose.

4. 'K' – 0x4B – Start/End/Query/On-Off Baseline

This is a command from the Base Unit, BE|USB adapter, or PC to the sensor to start, restart, end, or query the baseline status.

Key	K Value
1 Byte	1 Byte
'K' – 0x4B	

K Value	Meaning
0x01	start baseline gathering
0x00	stop baseline gathering
0xFF	cancel baseline gathering

If the sensor is not gathering baseline data, setting the K value to 0x01 will command the sensor to start gathering a baseline reading. Setting the sensor to baseline-gathering mode will put the sensor into a mode where the averaging time constant is approximately 1 sec.

If the sensor is currently gathering baseline data, the sensor is put in a "sensor busy" mode. This prevents the sensor from sending messages or receiving commands for the duration of the progress, with the exception of an interrupt baseline command. An error code is sent back with the value of "system busy".

If the baseline is gathering data, setting the K value to 0x00 will command the sensor to stop gathering a baseline reading. Upon completion of gathering a baseline, the baseline (B, b) value is updated, and the Averaging Time Constant is restored to the original value. Note that the B message is not automatically sent upon completion of calculation of the baseline.

If the baseline is not gathering data, setting the K value to 0x00 will have no effect.

If the baseline is gathering data, setting the K value to 0xFF will command the sensor to interrupt and cancel the gathering of a new baseline reading. Upon canceling, the baseline (B, b) value is not updated, and the Averaging Time Constant is restored to the original value.

If the baseline is not gathering data, setting the K value to 0xFF will have no effect.

Sending an empty-packet "K" command will return the "k" message, displaying the currently set values:

Key	K Value
1 Byte	1 Byte
'k' – 0x6B	

The returned values are either 0x01 (currently collecting baseline data) or 0x00 (not collecting baseline data).

5. 'L' – 0x4C – 'Sensor Check' Function Control

This is a command from the Base Unit, BE|USB adapter, or PC to the sensor to start, end, or apply the results of the 'Sensor Check' function. The Sensor Check function runs from within the sensor embedded software. The purpose of the 'Sensor Check' routine is to determine if the sensor is performing correctly, and, if necessary, reset the internal

sensor calibration. A one-byte value following the 'L' command provides the control for the 'Sensor Check' routine.

Key	L Value
1 Byte	1 Byte
'L' – 0x4C	

L Values	Meaning
0x00	Clear data.
0x01	Initiate Low cal cup measurement.
0x02	Initiate High cal cup measurement.
0x03	Use cal cup measurements to set new Variable Sensor Coefficients.
0xFF	Interrupt measurement and resume normal mode.

Note: values not defined above are illegal and ignored by the sensor.

Sending an empty 'L' command packet will return the 'l' message along with a data packet. The first Byte of the packet holds a status message. The meaning of the status messages are shown below:

l Values	Meaning
0x00	No data.
0x11	Busy with Low cal cup measurement.
0x12	Busy with High cal cup measurement.
0x13	Busy with setting new Variable Sensor Coefficients.
0x21	Low cal cup measurement was unstable.
0x22	High cal cup measurement was unstable.
0x31	Low cal cup measurement was stable but Combined Result failed.
0x32	High cal cup measurement was stable but Combined Result failed.
0x41	Low cal cup measurement was stable and Combined Result passed.
0x42	High cal cup measurement was stable and Combined Result passed.
0x53	Error - new Variable Sensor Coefficients could not be set.
0x63	New Variable Sensor Coefficients were successfully set.

If the 'Sensor Check' routine has just been started (either through the interactive keypad or the user interface software) and no calibration cup measurements have been initiated yet, sending an empty 'L' command to the sensor will result in an "l value" of 0x00 (No data) being returned.

If a calibration cup measurement is in progress when an empty 'L' command is sent to the sensor, a 'Busy' (0x11 or 0x12) "l value" will be returned to the sender. When a calibration cup measurement has been completed, sending an empty 'L' command to the sensor will result in one of 6 "l values" being returned. The returned "l value" can be used to determine the type of calibration cup that was measured ("low" or "high"), whether the standard deviation of the 10 calibration cup measurements was above ("unstable") or equal to or below ("stable") a threshold value. In the case that the

measurement was stable, the "l value" further indicates whether or not the sensor result, when compared to the result computed from the stored Calibration Cup Coefficients, had an absolute error that exceeded ("failed") or was equal to or below ("pass") a threshold value.

If calculation and saving of new Variable Sensor Coefficients is in progress when an empty 'L' command is sent to the sensor, a 'Busy' (0x13) "L value" will be returned to the sender. When new Variable Sensor Coefficients have been computed and saved, sending an empty 'L' command to the sensor will result in an "l value" of 0x63 being returned. When an error is encountered during the calculation or saving of new Variable Sensor Coefficients, sending an empty 'L' command to the sensor will result in an "l value" of 0x53 being returned.

In addition to the 1 Byte 'l value', 2 32-bit floats are returned in response to an empty packet "L" command. The first float value is the sensor response (in "Bug Units") measured on the calibration cup; the second float value is the stored sensor response. The "set new variable sensor coefficients" command should only be sent when "stable" measurements have been completed on both the Low and High Calibration Cups. When the cal cup measurement is interrupted (by sending an L value of 0xFF) the calibration cup measurement data will be cleared from sensor memory with no change to the Variable Sensor Coefficients. Sending an empty packet "L" query to the sensor after sending and "L" command with an "L Value" of either the 0x00 (clear data) or 0xFF (interrupt and resume normal mode), will result in an "l value" of 0x00 (no data) being returned.

Returned 'l' packet in 'normal' mode

Key	l Value	Measured Combined Result				Stored Combined Result			
1 Byte	1 Byte	4 Bytes				4 Bytes			
'l' – 0x6C		MSB			LSB	MSB			LSB

When the 'Sensor Check' routine is running in the sensor, all commands other than an 'L' command will be responded to with a "system busy" 1 byte error code.

6. 'M' – 0x4D – Data Request

This command is used to request a data packet from either the sensor or the Base Unit. Returns the sensor data output. The only format for the 'M' command is an empty 'M' command.

Key
1 Byte
'M' – 0x4D

Sending an empty-packet "M" command will return the "m" message, along with a data packet. The data packet has 6 components:

(1) Error Code (8-bit signed integer),
(2) Raw Sensor Measurement Result (in 'Bug Units') (32-bit floating point),
(3) Baseline-Corrected Measurement Result (32-bit floating point),
(4) User Calibrated Result (32-bit floating point),
(5) Growth Rate Constant (1/hours) (32-bit floating point),
(6) User Calibration Units (up to 11 ASCII bytes).

Key	Error Code	Raw Result				Base.-Corr. Result				User Cal Result				Growth Rate (1/hrs)				User Cal. Units
1 Byte	1 Byte	4 Bytes				4 Bytes				4 Bytes				4 Bytes				up to11 ASCII Bytes
'm' – 0x6D		LSB			MSB	LSB			MSB	LSB			MSB	LSB			MSB	…

Note: the total length of the data packet is variable due to the variable length of the 'User Cal Units' string. The 'User Cal Units' string includes a terminating null character as its last byte. Therefore, the maximum allowed number of non-null characters in the string is 10.

7. 'O' – 0x4F – On/Off Setting for Baseline Corr. and User Cal.

The 'O' command is used to turn On or Off Baseline Correction and User Calibration. Values are represented as 8-bit unsigned integers. Allowed values are 0 (Off) and 1 (On), with the default value being 0 (Off) for both functions. The first byte sets the On/Off state for Baseline Correction. The second byte sets the On/Off state for User Calibration. Sending the highest value for a byte (hex 'FF') will preserve the current setting in the byte. Values other than 0, 1, and F will be ignored.

Key	Baseline Correction On/Off	User Calibration On/Off
1 Byte	1 Byte	1 Byte
'O' – 0x4F		

Sending an empty-packet "O" command will return the "o" message, displaying the currently set values:

Key	Baseline Correction On/Off	User Calibration On/Off
1 Byte	1 Byte	1 Byte
'o' – 0x6F		

8. 'Q' – 0x51 – Base Unit Password Settings

You may wish to set up password-protected access to the keypad functions on the Base Unit. When in the locked state, entry to the Base Unit functions is enabled by entering a six-number value via the keypad. Only values from 1 to 4 are valid for each number. If the six numbers (ASCII) entered on the keypad match the currently-stored password, the system enters an unlocked state until the user exits the configuration menu. If the numbers entered do not match the currently-stored password, access to the configuration menus is denied, and returns to the normal display. The value of the Base Unit password is stored in the Base Unit. The default password is '111111'. In addition to changing the password via the keypad on the Base Unit, the password may be set through the 'Q' command:

Key	P-Value1	P-Value2	P-Value3	P-Value4	P-Value5	P-Value6
1 Byte	1 Byte	1 Byte	1 Byte	1 Byte	1 Byte	1 Byte
'Q' – 0x51						

The Q-command may also be used to reset and turn On or Off password protection by sending a 1-byte value for a Q-command. Sending a 0x00 turns password protection Off. Sending a 0x01 turns password protection On. Sending a 0x02 resets the password to "1,1,1,1,1,1" and turns Off password protection. Sending a 0x03 resets the password to "1,1,1,1,1,1" and turns On password protection. Note that the values for "1,1,1,1,1,1" are represented in hex as a six-element array of 0x31.

Key	Q-Command
1 Byte	1 Byte
'Q' – 0x51	

Sending an empty-packet "Q" command will return the "q" message, displaying the currently set status:

Key	q-Value
1 Byte	1 Byte
'q' – 0x71	

Valid return values are locked (0x00) or unlocked (0x01). This command is only useful for BE2100 and BE2400 base units; in the case of the BE|USB adapter no keypad access is provided, so this command serves no purpose.

9. 'R' – 0x52 – Range for Analog Output

Sets the range for the analog output terminals of BE2100 and BE2400 Base Units. Values are represented as 8-bit unsigned integers. Allowed values are 0-5 with a default value of '4'. The table below shows the correspondence between the integer representation and the range value:

Integer Representation	Range Value

0	0.01
1	0.1
2	1.0
3	10.0
4	100.0 (default)
5	1000.0

The first byte is the range for Analog Out 1. The second byte is the range for Analog Out 2 (BE2100 Base Units only). Sending the high value (hex FF) for a byte will preserve the current setting in that byte. Byte values other than 0, 1, 2, 3, 4, 5 and FF are ignored.

Key	AO1&2 Range	
1 Byte	1 Byte	1 Byte
'R' – 0x52	AO1	AO2

Sending an empty-packet "R" command will return the "r" message, displaying the currently set values:

Key	AO1&2 Range	
1 Byte	1 Byte	1 Byte
'r' – 0x72	AO1	AO2

The above description applies only to the "standard" configuration where a sensor is connected to a BE2100 base unit. If a BE2400 (multiplexing) base unit is used instead, only one analog output is available for each sensor (AO1); changing the range setting for AO2 has no effect. If the sensor is connected via a BE|USB adapter no analog output is available, so the range command serves no purpose.

10. 'S' – 0x53 –Sensor Serial Number

This command reads the Sensor Serial Number. The field data is represented as a 32-bit unsigned integer. Sending an empty-packet 'S' command will return the 's' message, displaying the currently set values. It is also sent automatically when the device is first powered up (following the send of the Base Unit or BE|USB embedded software version data).

Key	Sensor S/N			
1 Byte	4 Bytes			
's' – 0x73	MSB			LSB

11. 'T' – 0x54 – User Calibration Units

Null-terminated string of up to 11 characters describing the user-calibration units (e.g. 'mg/L e coli'). Default setting is "Cal Units". Non-printable ASCII characters are ignored.

Key	User Cal Units

1 Byte	(up to) 11 ASCII Bytes						
'T' – 0x54				…			

Sending an empty-packet "T" command will return the "t" message, displaying the current string:

Key	User Cal Units						
1 Byte	(up to) 11 ASCII Bytes						
't' – 0x74				…			

The last byte is to be used for the null-termination character, so the maximum useful string length is 10 characters. Note: The response string contains 1 more character than the command string; this last response character should be ignored.

12. 'U' – 0x55 –User Calibration Coefficients

Six 32-bit floating-point values and one unsigned integer: offset, linear coeff, quadratic coeff, cubic coeff, MinX, and MaxX, and Transform Method. Min and Max X are the minimum and maximum values of the Sensor Measurement Results used in generating the calibration coefficient. These values are useful for determining whether the calibration is being extrapolated beyond the range of the calibration data. Transform Method is either linear (value = 0) or log (value = 1). Defaults are defined as follows: [0.00, 1.00, 0.00, 0.00, 0.00, 0.00]

Key	Offset				Linear Coeff			
1 Byte	4 Bytes				4 Bytes			
'U'– 0x55	LSB			MSB	LSB			MSB

Quadratic Coeff				Cubic Coeff			
4 Bytes				4 Bytes			
LSB			MSB	LSB			MSB

MinX				MaxX				Transform Method
4 Bytes				4 Bytes				1 Byte
LSB			MSB	LSB			MSB	

(the coefficients appear as a continuous message; this table has been formatted to fit on the page).

Sending an empty-packet "U" command will return the "u" message, displaying the currently set array:

Key	Offset				Linear Coeff			
1 Byte	4 Bytes				4 Bytes			
'u'– 0x75	LSB			MSB	LSB			MSB

Quadratic Coeff				Cubic Coeff			
4 Bytes				4 Bytes			
LSB			MSB	LSB			MSB

MinX				MaxX			
4 Bytes				4 Bytes			
LSB			MSB	LSB			MSB

(the coefficients appear as a continuous message; this table has been formatted to fit on the page).

13. 'V' – 0x56 – Sensor Embedded Software Version

This command reads the version of the embedded software that exists in the sensor. The data field is comprised of a 32 bit floating point value. The only format for the 'V' command is an empty 'V' command.

Key
1 Byte
'V' – 0x56

Sending an empty-packet 'V' command will return the 'v' message, displaying the current setting:

Key	Sensor S/W version			
1 Byte	32 bits			
'v' – 0x76	LSB			MSB

14. 'W' – 0x57 – Growth Rate Window

The 'W' command determines the time window (in seconds) to be used when estimating the exponential growth rate contant. The data field is comprised of an unsigned integer16. Any value that is below the Averaging Time Constant (see the 'A' command) is set to the Averaging Time Constant for windowing. Allowed values are: 60, 120, 240, 480, 960, and 1920. The default value is 480.

Key	Slope Window	
1 Byte	2 Bytes	
'W' – 0x57	MSB	LSB

Sending an empty-packet "W" command will return the "w" message, displaying the current setting:

Key	Slope Window	
1 Byte	2 Bytes	
‘w’ – 0x77	MSB	LSB

15. ‘@’ – 0x40 – Base Unit Embedded Software Version

This command reads the version of the embedded software that exists in the Base Unit. This data is sent automatically when the device is first powered up. The data field is comprised of a 32 bit floating point value. The only format for the ‘@’ command is an empty ‘@’ command.

Key
1 Byte
‘@’ – 0x40

Sending an empty-packet ‘@’ command will return the ‘2’ message, displaying the current setting:

Key	Base Unit S/W version			
1 Byte	32 bits			
‘2’ – 0x32	LSB			MSB

16. ‘#’ – 0x23 –Base Unit Serial Number

This command reads the Base Unit Serial Number. The field data is represented as a 32-bit unsigned integer. Sending an empty-packet ‘#’ command will return the ‘3’ message, displaying the currently set values.

Key	Base Unit S/N	
1 Byte	4 Bytes	
‘3’ – 0x33	MSB	LSB

Appendix IV. Example Procedure for Calibrating the Analog Outputs on a BE2100 or BE2400 Base Unit

The analog outputs (AO) on BE2100 and BE2400 base units provide current outputs that are proportional to biomass (BE2100 and BE2400) or growth rate (BE2100 only). The nominal range of the current is 4 to 20 mA. However, due to component variation, the actual minimum and maximum currents vary somewhat between base units. Therefore, when using the AO outputs, it recommended that the actual minimum and maximum currents be measured and used as calibration inputs for the AO reading device (such as an analog-to-digital converter on your bioreactor controller). The following example provides a step-by-step method for measuring the minimum and maximum AO currents.

Tools needed:

- DC Voltmeter (with measurement range of ~1 mV to at least 10 V).
- Miniature flat-head screw driver (blade size ~2.5 mm).
- Analog output terminal block (4 or 8 position, respectively, as provided with BE2100 and BE2400 base units).
- 500 Ω resistor, 1% precision (as provided with BE2x00 base units).
- low and high calibration cups (as provided with BE2100 sensors).

Procedure:

1. Set the averaging time constant on the base unit to zero (see **Introduction to the BE2x00 Base Unit Functions** for a step-by-step procedure).
2. Set the Range for the analog output you wish to calibrate to 100 (see **Appendix II, Key Pad Communication**, section 1.v, for further details).
3. Turn Off both Baseline Correction and User Calibration and then exit back out of the configuration menu. The bottom line of the base unit should now show read in "Bug Units" (indicating that User Calibration is Off), and the letter "C" should not be displayed at the end of the line (indicating that Baseline Correction is Off) (see **Appendix II, Key Pad Communication**, for further details).
4. Remove all of the magnetic adapter sleeves from the sensor. Place the "Low" calibration cup over the front of the sensor. Make sure that the arrow marked on the calibration cup is pointing towards the arrow marked on the sensor. Connect the buckle components attached to opposite ends of the strap. Cinch the sensor against the calibration cup by pulling on the free end of the strap that protrudes from the buckle. Next close the latch on the back of the sensor --- this step should provide the final pressure needed to firmly seat the sensor against the calibration cup.
5. The biomass reading on the bottom line of the base unit display should now read "0.00 Bug Units".
6. Using a miniature flat-head screwdriver, attach a 500 Ω (1% precision) resistor across the "+" and "-" terminals of the analog output terminal block, for the analog output that you wish to calibrate.
7. Plug the analog output terminal block into the mating connector at the rear of the base unit.

8. Using the Voltmeter, measure the DC voltage across the 500 Ω resistor. Record this as V(low).
9. Replace the low calibration cup with the high calibration cup. Secure the high calibration cup against the sensor, as detailed in step 4 above. The biomass reading on the bottom line of the base unit should now be greater than 100 Bug Units.
10. Using the Voltmeter, measure the DC voltage across the 500 Ω resistor. Record this as V(high).
11. Return the sensor averaging time constant, range, baseline correction, and user calibration settings to their prior settings.
12. Convert V(low) and V(high) from voltages (V) to currents (mA) by multiplying by 2:
    I(low) = 2*V(low)
    I(high) = 2*V(high)
13. Use I(low) and I(high) as calibration inputs for your analog reading device (e.g. bioreactor controller). This step will vary depending on the control software you are using, but typical linear calibration inputs are "offset" and "span" values in units of current and corresponding biomass units. The table below shows the correspondence between offset and span and the values you just measured.

Table 1. Example Linear Analog Calibration Inputs

	Current (mA)	**Biomass**
Offset	I(low)	0.00
Span	I(high)-I(low)	BE2x00 base unit Range setting

Note that the Range value you use for the Biomass Span should be the Range setting that you will select during your bioreactor run (not the Range setting just used during the calibration procedure). If you change the range setting on the BE2x00 base unit, you will also need to update the calibration in your control software. Also, the table above assumes that baseline correction will be applied to the BE2100 sensor result so that when media alone is measured, the biomass reading is zero. If baseline correction is not applied and the biomass reading for media alone is not zero, you should use this baseline reading as the Biomass Offset and subtract this value from the span. However, baseline-correction via the BE2x00 instrument is generally recommended, since this can be applied without having to update the calibration inputs for your control software.

Appendix V. Trouble-Shooting

Sensor Trouble-Shooting

Observation	Possible Causes	Suggested Remedies
Disagreement between BE2100 sensor and offline reference measurement	(1) Incorrect calibration selected. (2) Incorrect baseline. (3) Interference from objects within the bioreactor. (4) Cell lysates are contributing significantly to the measured optical reflectance.	(1) See ***User Calibration***. (2) Collect a baseline reading on the media alone, prior to inoculation. See ***Setting the Baseline***. (3) See ***Setting up for a Bioreactor Run: Step 4***. (4) See ***Principles of Operation***.
Sensor Check test failed.	(1) Incorrect calibration cups were used. (2) Dirt on window or calibration cups. (3) Laser aging. (4) Laser burned out due to excessive temperature (e.g. left on bioreactor during sterilization cycle).	(1) Make sure that the last 2 digits of the calibration cup serial numbers match with that of the sensor. (2) Clean the Sensor Window and/or Verification Cup. See ***Verification of Sensor Performance***. (3) At the end of the Sensor Check procedure update the sensor coefficients. See ***Verification of Sensor Performance***. (4) The sensor will need to be returned to BugLab so that the laser can be replaced and the sensor re-calibrated.
Biomass readings are not stable.	(1) Sensor position needs to be optimized. (2) The sensor averaging time constant needs to be optimized.	(1) See ***Setting up for a Bioreactor Run: Step 4***. (2) Set the sensor averaging time constant to the highest value allowed by the growth rate of the culture. See step 6A of ***Setting Up and Configuring***.

Sensor Calibration Trouble-Shooting

Observation	Possible Causes	Suggested Remedies
"Extrapolating Cal"	The displayed biomass is outside the range of the user-generated calibration.	This message is provided for informational purposes. No action is required.
"Below range"	The displayed biomass is below the range of the internal sensor calibration.	This message is provided for informational purposes. As the biomass increases, this message will automatically disappear.
"User cal error"	Calibration transform is set to Log-Log, and negative or zero-valued data encountered.	(1) Make sure the baseline is correctly set. AND (2) Wait for the biomass to increase above 0. OR (3) Switch to the linear transform method. See ***Editing, Generating, and Saving a Calibration***.

Base Unit Trouble-Shooting

Observation	Possible Causes	Suggested Remedies
The base unit keypad is un-responsive	Base unit is in remote mode.	(1) Exit out of the BE2x00 Virtual Instrument software. (2) If the keypad is still un-responsive, turn the base unit off and back on again.
The base unit is requesting a password, but the password is lost.	A prior user has turned on password protection of the configuration settings.	See ***Password Locking and Unlocking***.
Multiple sensors are plugged into the BE2400 base unit, but only 1 sensor result is being displayed on base unit screen.	Scrolling mode is Off.	(1) If operating in local mode, use the keypad to set the display mode to Scrolling. See ***Introduction to the BE2400 Base Unit Functions***. (2) If operating in remote mode, go to the Device Configuration window and check the "Base Unit Display Scrolling" settings.
Analog output is not responsive to changes in sensor readings.	Range setting is not optimal.	Make sure the AO range setting matches with the maximum anticipated biomass reading. See ***Working with the BE2100 and BE2400 Base Units: Analog Output***

BE2x00 Software Communication Trouble-Shooting

Observation	Possible Causes	Suggested Remedies
BE2100 sensor not detected by *BE2x00 Virtual Instrument* software.	(1) Sensor, USB or RS-232 cable not connected. (2) Base Unit is in local mode. (3) RS-232 and USB communications are in conflict. (4) USB driver was not correctly installed. (5) BE2x00 device not recognized by a USB hub.	(1) See ***Setting up and Configuration***. (2) Exit out of the configuration menus. (3) If communicating via RS-232, make sure the USB cable is unplugged from the base unit. If communicating with a BE2100 base unit via USB, make sure the comm port setting is "USB". See ***Setting Up and Configuring***. (4) See ***USB Driver Trouble-Shooting*** at the end of this table. (5) Re-boot your computer.
Computer communication with the BE2x00 device was interrupted.	(1) The computer went into sleep mode. (2) Power to the BE2x00 device was interrupted. (3) Connecting through a USB hub device.	(1) See Step 1 of ***Setting Up and Configuring***. (2) Consider connecting the BE2x00 device to a power source that can provide uninterrupted power (e.g. battery back-up). (3) If possible, connect your BE2x00 device directly to a USB or RS-232 port on your computer, rather than connecting through a USB hub (which we have found to be less reliable).

USB Driver Trouble-Shooting

If you are connecting the BE2x00 instrument to a computer via USB, but the instrument is not recognized by the User Interface software, the USB driver software may not have been installed correctly. The following procedure describes how to check and, if necessary, re-install the USB communication driver software. This procedures assumes that you have already installed the User Interface software (if not, first follow the steps in ***Software Installation***).

1) Check to make sure that the driver was installed properly by checking the status in Device Manager:
   a. Make sure that your BE2x00 instrument is plugged into a USB port on your computer.
   b. Press the windows Start button and choose Control Panel.

c. Locate and double-click on the “Device Manager” icon.
d. Scroll down the devices to “Ports (COM & LPT)” and view the listed devices (by clicking on the windows expansion arrow).
e. One of the listed ports should be “USB Serial Port (COMx)”, where x is an integer (e.g. “USB Serial Port (COM8)”). If no such device is listed, skip to step 3 below.
f. Right-click on the USB Serial Port and select “Properties”.
g. Click on the “General” tab. The “Device status” window should be displaying “This device is working properly”. If not, skip to step 2 below.
h. Click on the “Driver” tab. The driver that is listed should be FTDI version 2.12.0.0, or higher. If it is not, proceed to step 2 below.

2) If the device is not working properly or the wrong FTDI version is listed, uninstall the driver software:
   a. Disconnect the USB cable from the BE2x00 instrument and the computer.
   b. Under the “Driver” tab, select “Uninstall”.
   c. In the Uninstall window that pops up, click on “Delete the driver software for this device” and hit “OK”.
3) Install the driver:
   a. If you haven’t done so already, disconnect the USB cable from the BE2x00 instrument and the computer.
   b. Go to the root directory where the BE2x00 software was installed. By default this is: “C:\Program Files\BugLab\BE2x00”.
   c. Within this folder double-click on the file named “CDM v2.12.00 WHQL Certified.exe”.
   d. In the pop-up window answer “Yes” to allow changes to your computer.
   e. A window entitled “FTDI CDM drivers” should open. Select “Extract”.
   f. The “Device Driver Installation Wizard” should automatically start. Select “Next” and wait for a confirmation that the driver was successfully installed. Select “Finish” to exit out of the Installation Wizard.
4) Plug in the BE2x00 instrument:
   a. Make sure a BE2100 sensor is connected to your base unit or BE|USB adapter.
   b. If you are working with a BE2100 or BE2400 base unit, make sure it is plugged in, turn on the base unit, and wait for the sensor to be detected.
   c. Connect a USB cable to the BE2x00 instrument and to the computer.
   b. You should see a windows message: “Installing device driver”.
   c. Wait until a new message appears: “Device driver software installed successfully”.
5) Start up the BE2x00 Virtual Instrument software and wait for the list of detected BE2x00 devices to finish updating

END